



**THE MICROBIOLOGY OF  
CELLULOSE, HEMICELLULOSES  
PECTIN AND GUMS**

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**THE MICROBIOLOGY OF  
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PECTIN AND GUMS**

**By A. C. THAYSEN AND  
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## P R E F A C E

IN their investigations on the microbiological destruction of cellulose the writers have often felt the need of a comprehensive account of the information available on this and the allied subjects of the microbiological changes occurring in hemicelluloses, pectin, and gums.

In the present treatise an attempt has been made to fill this gap and to give at least a broad outline of the more essential literature already in existence. The treatise has been compiled from the point of view of the research worker who desires to know in what direction his efforts may most profitably be directed within this important subject of the natural and artificial decomposition of vegetable tissues, which, as knowledge increases, acquires added theoretical and practical importance.

In order to avoid misunderstanding it should be mentioned perhaps that the term micro-organisms has been adopted to designate all microscopic organisms whether they belong to the animal or to the vegetable kingdom, and that colloquially the word 'bacteria' has been used for all the rod-shaped schizomycetes belonging to the eubacteriales. Where a distinction in the Latin nomenclature has been necessary among the bacteria, the system followed by Lehmann and Neumann and by Bergey has been adhered to and a spore-producing rod termed 'Bacillus', a non-spore-forming 'Bacterium'.

In placing their treatise before a wider public the writers wish to express their sincere thanks to Captain A. P. H. Desborough, C.B.E., R.A., Superintendent of the Royal Naval

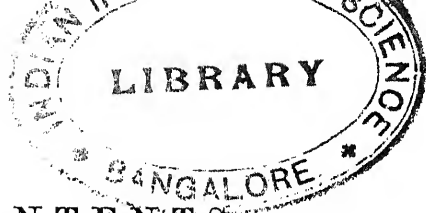
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They also greatly appreciate the permission granted them by the Lords Commissioners of the Admiralty to publish their treatise.

HOLTON HEATH,

*September, 1925.*



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PART ONE

CELLULOSE, HEMICELLULOSES  
PECTIN AND GUMS



## CHAPTER I

### OCCURRENCE AND PROPERTIES

BEFORE attempting to give an account of the microbiology of cellulose, hemicelluloses, pectin, and gums, it is necessary briefly to refer to the occurrence of these substances in nature and to give an outline of their chemical and physical properties in so far as they have a bearing on the subject dealt with in the following chapters.

*Cellulose* was first isolated by Payen<sup>1</sup> in 1839 in an endeavour to purify the component parts of the cell walls of plants. After successive treatments of such tissues with solvents such as water, alcohol, acids, and alkalies, Payen obtained in every case a residue which on chemical analysis could be shown to contain the elements of carbon, hydrogen, and oxygen in the same proportions in which they are present in starch  $(C_6H_{10}O_5)_x$ . He termed this residual substance cellulose. In addition to 'pure resistant cellulose' Payen's cellulose must have contained other compounds, notably hemicelluloses, since the account given by Dumas, Pelouze, and Brongniart<sup>2</sup> of Payen's work specifically mentions the perisperm of *Phytalephas macrocarpa* as an example of cellulose. Gilson<sup>3</sup> appears to have been the first to limit the term cellulose to that resistant part of the cell wall which on hydrolysis breaks down to form glucose.

It is now known that among the phanerogams, cellulose constitutes from 30 to 50 per cent. of all lignified cell structures, and that in substances such as cotton-wool the cellulose represents about 90 per cent. of the total weight of the air-dry material. Among the lower plants cellulose is regularly met with in the tissues of ferns (Gilson<sup>3</sup>). In the cell wall of the mosses it is probably present (Czapek<sup>4</sup>),



and among the higher algae it has been reported present in the *Florideae* and the *Phaeophyceae* by van Wisselingh<sup>5</sup>. Its presence in the tissues of lower algae, fungi, and bacteria is still problematical. If present, it is not as important in these cases as a cell structure as it is in the higher plants, and its functions have been largely taken over by hemicelluloses and chitinous substances.

In exceptional cases it may be met with in the animal kingdom, where Schmidt<sup>6</sup> isolated it from the mantle of the *Tunicata*.

The hemicelluloses, which are perhaps even more widespread in plant tissues than cellulose, particularly among the lower plants, have been less thoroughly investigated than cellulose. They were first studied as a separate group by Schulze<sup>7</sup>, who found that the cell membranes of plants contain certain insoluble compounds, the hemicelluloses, which on boiling with weak sulphuric acid are readily hydrolysed and converted into soluble carbohydrates, usually other than glucose.

Schulze and his collaborators found hemicelluloses in the seeds of many plants, acting in this case as a reserve food. Other hemicelluloses were found in the lignified tissues of phanerogams, their function here being to strengthen the cell walls. The amount of hemicelluloses found in phanerogamous tissues varied very considerably. Schulze<sup>8</sup> gives figures ranging from 2.36 per cent. to 50.83 per cent. No figures are available for the hemicellulose contents of ferns, mosses, algae, fungi, and bacteria. They are probably in no case negligible and may in many cases be appreciable.

The pectic substances of plant tissues, which in this volume are dealt with as a separate entity, though chemically speaking they may not be so, are less important quantitatively than the two former groups. Pectin occurs in all phanerogamous tissues as the main constituent of the middle lamella (Payen<sup>9</sup>), and it is perhaps present with cellulose in the actual cell walls of certain fleshy roots. In turnips, for instance, the quantity of pectin present is stated to be about 18 per cent., in oranges 26 per cent., and in apples 6 per cent. (Clayson, Norris, and

Schryver<sup>10</sup>). How far pectin occurs in the lower plants is not yet clear; Czapek<sup>4</sup> regards its presence as probable.

Under the name of gums a variety of substances have been described which differ considerably from one another both chemically and physiologically. Originally the name gum was applied to substances exuded by certain phanerogams, for instance, the *Mimosaceae* and the *Amygdalaceae*, after injury of their tissues. These exuded gums are not a normal constituent of the tissues but a decomposition product of the cell walls (Czapek<sup>4</sup>), and sometimes, for instance in the case of tragacanth gum, show traces of the normal cell structure (von Mohl<sup>11</sup>). The suggestion has been put forward by several investigators, Prillieux and Delacroix<sup>12</sup>, Aderhold<sup>13</sup>, Greig Smith<sup>14</sup>, and Groenewege<sup>15</sup>, that the exudation of gums of higher plants is due to the infection of their tissues by micro-organisms. This view, however, has not been generally accepted (Rathay<sup>16</sup>). Sorauer<sup>17</sup> some time ago suggested a new theory for their formation. Further information on this question may be found in text-books dealing with the microbiology of plant diseases.

The substances, carrageen obtained from the alga *Chondrus crispus*, agar-agar from *Gracilaria lemaneoides*, and wood gum from lignified phanerogams, do not belong here, but to the hemicelluloses.

Reference should be made to another type of gum, the group of gum-resins which are mixtures of gums and aromatic compounds and are exuded by certain plants. As examples may be mentioned myrrh from *Commiphora* species, olibanum from species of *Boswellia*, and asafoetida from *Ferula narthex* and *Ferula foetida*. Little is known either of the origin of these gum-resins (Czapek<sup>4</sup>) or of their functions. The fact that they contain aromatic compounds possibly indicates that they act as protective substances.

Turning to the chemistry of cellulose, hemicelluloses, pectin, and gums, it was mentioned that the lignified tissues of the higher plants contain from 30 per cent. to 50 per cent. of cellulose. Chemically pure cellulose does not occur in nature, but the seed hairs of *Gossypium barbadense* and allied species,

and the bast fibres of *Linum usitatissimum* contain as much as 90 per cent. of pure cellulose in air-dried samples.

Pure cellulose exhibits remarkable resistance to the action of chemicals and can be isolated from plant tissues only by the use of drastic chemical treatment. A description of the methods used for its isolation would hardly come within the scope of this inquiry: readers interested in the subject are referred to existing text-books on paper manufacture. Nor is it possible to give a detailed account of the present position of the chemistry of cellulose and of the various views held of the chemical constitution of the cellulose molecule. It will be necessary, however, to touch upon some aspects of these problems, since they have an immediate bearing on subjects which will be dealt with in the following chapters.

When cellulose is immersed in concentrated sulphuric acid it gelatinizes and gradually goes into solution (Braconnot<sup>18</sup> and Ost and Wilckening<sup>19</sup>). The solution contains cellulose sulpho-esters which are decomposed on boiling in dilute sulphuric acid, yielding large quantities of fermentable carbohydrates. Up to 96.3 per cent. of the theoretical yield of glucose was obtained by Willstätter and Zechmeister<sup>20</sup> on dissolving cellulose in concentrated hydrochloric acid containing 41 to 42 per cent. of hydrogen chloride. Here, too, the cellulose gelatinizes when immersed in the acid; subsequently it dissolves to form a viscous liquid which after fifteen minutes' standing changes to a thin fluid. With an acid containing 41.4 per cent. to 42 per cent. of hydrogen chloride 15 per cent. of cellulose may thus be dissolved. Irvine and Soutar<sup>21</sup> have obtained similar yields. Both they and Monier-Williams<sup>22</sup> isolated the product of hydrolysis and found it to consist of glucose only. The conclusion may therefore be drawn that cellulose is composed entirely of glucose residues.

The method described by Willstätter and Zechmeister for the saccharification of cellulose has acquired considerable interest, as it forms a basis for the isolation of lignin from lignified plant tissues. On treating such tissues with an acid containing 42 per cent. of hydrogen chloride, the cellulose

is dissolved out while the lignin remains behind structurally unchanged.

Another acid which dissolves cellulose and forms an ester with it is acetic acid in the anhydrous form and in the presence of sulphuric acid. By the action of acetic anhydride Franchimont<sup>23</sup> first obtained the acetate of what appeared to be glucose, but which was found subsequently to be an acetate of a disaccharide. Skraup<sup>24</sup> and Skraup and König<sup>25</sup>, who investigated this substance, termed it cellose or cellobiose, and showed that it could be converted into glucose by hydrolysis with dilute acids. The yields of cellobiose obtained by these investigators were very small, but yields of 60 per cent., and more, of those theoretically possible, assuming cellulose to be composed entirely of cellobiose groups, are reported by Karrer<sup>26</sup>. Pringsheim<sup>27</sup> considers that the violent reaction necessary for the preparation of cellobiose is responsible for the low yields and is inclined to regard the cellulose molecule as built up entirely of cellobiose groups. Irvine and Hirst<sup>28</sup>, on the other hand, regard the cellulose molecule as composed of a trianhydroglucose, a constitution which takes into account that a maximum of 60 per cent. of cellobiose is obtainable from cellulose. On the other hand, Herzog's<sup>29</sup> X-ray spectrographic investigations of cellulose indicate that the group  $(C_6H_{10}O_5)_4$  is regularly repeated in the cellulose molecule. The justification of Herzog's conclusions are denied by Hess, Weltzien, and Messmer<sup>30</sup>, who consider the basic unit of the cellulose molecule to be an anhydroglucose  $(C_6H_{10}O_5)_1$ . Cellobiose, according to them, is not to be regarded as a decomposition product of cellulose but as an autocondensation product of the basic anhydroglucose of the cellulose molecule.

It has already been mentioned that the dissolution of cellulose in sulphuric and hydrochloric acid is preceded by the gelatinization of the carbohydrate. A gelatinization of cellulose can also be brought about by its treatment with other reagents such as strong alkali solutions, solutions of zinc chloride, and solutions of certain salts of thiocyanates (Williams<sup>31</sup>). The first reagent for the gelatinization and dissolution of cellulose was described by Schweizer<sup>32</sup> in 1857.

This reagent consists of a solution of cupric oxide in aqueous ammonia. Under the name of Schweizer's reagent it is commonly used for dissolving cellulose. From solutions of cellulose in cuprammonium, Gilson<sup>3</sup> collected spherocrystals of cellulose, the first observation made that cellulose may occur in crystalline form. It is but comparatively recently (Herzog and Jancke<sup>33</sup>) that further information has been forthcoming showing that cellulose occurs in nature in the form of crystals, and not as a colloid as had previously been generally assumed. In the ordinary way cellulose is precipitated from Schweizer's reagent as a fine precipitate, a form in which it is frequently used in the microbiological technique for the isolation of cellulose-decomposing micro-organisms.

Cellulose previously acted upon by strong solutions of sodium hydroxide is used in the viscose process for the preparation of artificial silk. Here carbon bisulphide reacts with the alkali-treated cellulose to form the sodium salt of the xanthogenic ester of cellulose which is soluble in water at ordinary temperatures (Cross and Bevan<sup>34</sup>). The preparation of xanthate of cellulose has been utilized by Balls<sup>35</sup> for the study of the development of the seed hairs of the cotton plant, and by Fleming and Thaysen<sup>36</sup> and others for the study of the changes occurring in cellulose hairs and fibres after their exposure to destruction by micro-organisms or chemical agents.

*Lignocellulose*, the vasculose of Fremy<sup>37</sup>, does not give the usual reactions for cellulose, for instance the purple coloration with zinc chloride and iodine. Instead, it is coloured purple by a solution of phloroglucin in hydrochloric acid (Wiesner quoted by von Höhnelt<sup>38</sup>). This reaction with phloroglucin has been ascribed to the presence of vanillin in the lignified cellulose, but was shown by Czapek<sup>4</sup> to be due to the presence of another aldehyde, hadromal. Another reaction for lignocellulose is the yellow coloration produced by aniline salts (Wiesner, *loc. cit.*). Lignocellulose is regarded by Cross and Bevan<sup>39</sup> as an ester of lignin and cellulose, that is as a chemical combination of these substances. Other workers, notably Wislicenus<sup>40</sup>, König<sup>41</sup>, and Lehne and Schepmann<sup>42</sup>,

regard lignocellulose as an adsorption compound of cellulose with lignin and other encrusting substances. Wislicenus's theory of the formation of lignified tissues is particularly interesting. He regards the primary cell walls of all plant tissues as built up of cellulose. On this substance the gradually growing colloidal molecules of lignin, hemicelluloses and other encrusting compounds become deposited, owing to their increasing size and to the catalytic action of the cell wall. The adsorption theory of lignocellulose formation is supported by the fact that the cellulose of lignified tissues can be removed without interference with their structure, and that the lignin can be similarly eliminated, leaving a mould of the tissues consisting of cellulose.

The chemical constitution of lignin is still unknown. Some authors, e. g. Klason<sup>43</sup> and Schrauth<sup>44</sup>, favour the view that lignin possesses an aromatic structure, and Klason has found indications of the existence of two different compounds, an  $\alpha$ -lignin and a  $\beta$ -lignin, in the substance usually described as lignin; they are stated to be present in almost equal quantities. Willstätter and his collaborators<sup>45</sup> regard lignin as related to the carbohydrates, since the decomposition products obtained from it on treatment with hydriodic acid and phosphorus can also be obtained from xylan and cellulose by the same treatment. Dorée<sup>46</sup> in his survey of recent literature on the subject of the constitution of lignin expresses the view that its structure bears resemblance to that of cholesterol and phytosterol. The lignin used in the investigations referred to above was obtained either by treatment of wood with concentrated hydrochloric acid or from the lignin obtained in the sulphite process of paper manufacture. Such lignin, however, may have undergone changes during its preparation which may have affected its structure (Riefenstahl<sup>47</sup>). It would be a problem of absorbing interest to determine whether the enzymatic resolution of the cellulose in lignified tissues could be so conducted as to yield a less damaged lignin molecule.

Before leaving the subject of lignin it should be mentioned that Hägglund<sup>48</sup> considers that lignin contains from 6.3 to

6.6 per cent. of chemically-bound pentosan. This view is contradicted by Heuser<sup>49</sup>, however, who contends that the pentosan content of lignin is far too variable for it to be chemically bound.

Another substance to which a few lines must be devoted is *suberin*. This substance, which was first studied by Chevreul<sup>50</sup>, is found in the epidermis, bark, and corky substances of plants. It has been shown to contain large percentages of fatty acids, termed cerine by Chevreul. Gilson<sup>51</sup> records as much as 44 per cent. of fatty substances in suberin. How far cellulose is also present has not yet been definitely proved. Zemplén<sup>52</sup> reports that a substance is present which resembles cellulose, but which does not yield cellobiose octacetate. Pentosans have been reported present in suberin by Counciler<sup>53</sup>. Should it be finally proved that cellulose forms a substantial part of the walls of cork cells, it might be justifiable to regard suberin as cellulose impregnated with various fatty substances, very much as lignocellulose may be regarded as cellulose encrusted with lignin.

The *hemicelluloses*, by which name certain cell-wall substances are described, do not contain cellulose. It has already been mentioned that they may be completely converted into monoses, such as mannose, galactose, arabinose, or xylose. The hemicelluloses, therefore, are condensation products of these monoses. In the form of mannose and galactose condensation products, that is as mannans and galactans, they occur in coniferous woods and in the seeds of many plants, for instance in the seed of the date palm *Phoenix dactylifera*, and in that of *Phytalephas macrocarpa*, in the latter case as 'vegetable ivory'. The hemicelluloses which yield arabinose and xylose, and which are known as pentosans, usually serve as structural support for the cellulose in the cell walls. Xylan, particularly, is found in all lignified tissues in various quantities. On the decay of these tissues the pentosans may accumulate in the soil, where they are gradually broken down by certain micro-organisms.

Hemicelluloses in the form of methyl pentosans occur in

small quantities in most lignified tissues (Sebelien quoted by Czapek <sup>4</sup>).

The chemistry of *pectin* is in many ways as obscure to-day as it was when this substance was first isolated by Braconnot <sup>54</sup>. Its practical importance makes it necessary to touch upon some aspects of this subject. It can be regarded as proved that pectin occurs in considerable quantities in the cell walls of many fruits and fleshy roots. Tschirch <sup>55</sup> and Rosenberg <sup>56</sup> have demonstrated its presence in such tissues by a staining reaction, for instance with methylene blue or with ruthenium red, for which dyes pectin has a great affinity. With the same dyes pectin can be detected in the middle lamellae of all tissues and as a covering of intercellular cavities. Where it occurs between the individual fibres of flax it is very resistant to microbiological destruction. Störmer <sup>57</sup> has suggested that it might be present in a lignified form in this case.

Pectin, which most authors regard as insoluble, is sometimes described as proto-pectin (Tschirch <sup>55</sup> and von Fellenberg <sup>58</sup>), and sometimes as pectinogen (Schryver and Haynes <sup>59</sup>), or pectose (Carré <sup>60</sup>). The proto-pectin is said to become soluble in the cell sap on the ripening of fruits, and it can be rendered soluble by prolonged boiling with water or alcohol or by treatment with dilute acids (Carré <sup>60</sup>), or with salts of acids which form insoluble calcium salts, such as ammonium oxalate, ammonium tartrate, or sodium carbonate (Clayson, Norris, and Schryver <sup>10</sup>). The soluble compound from ripe fruits is termed pectin by von Fellenberg <sup>58</sup> and cytopectic acid by some English investigators. Von Fellenberg's pectin, which is neutral, is claimed to be the methyl ester of pectic acid. It is saponified by treatment with alkali in the cold and thereby yields methyl alcohol, amounting to some 10 per cent. of the total pectin or cytopectic acid.

Clayson, Norris, and Schryver <sup>10</sup> deny that the methyl alcohol which may be obtained by the treatment of pectinogen with alkali in the cold has any relationship to pectin. Nor do they obtain such large quantities of methyl alcohol as von Fellenberg, but only 0.2 to 0.3 per cent.

The pectic acid, or cytopectic acid of Clayson, Norris, and



Schryver, like von Fellenberg's pectin, forms a colloidal solution with water and may be precipitated as a gel from this solution by the addition of alcohol. It can be precipitated also by the addition of N/10 Na(OH), subsequent neutralization with acetic acid, and the addition of calcium chloride. Carré and Haynes<sup>61</sup> regard the precipitate thus formed as the calcium salt of pectic acid and have evolved a method for the quantitative determination of pectin on the basis of these observations.

More recently a paper has been published by Tutin<sup>62</sup> in which it is maintained that the insoluble proto-pectin or pectinogen is identical with the soluble pectin or cytopectic acid. The reason for the apparent insolubility of the pectin of unripe fruits, and presumably of other tissues containing proto-pectin, is ascribed by Tutin to the fact that the tissues containing the proto-pectin do not allow sufficient water to permeate for the solution of this sparingly soluble substance. Tutin finds that where the tissues are thoroughly disintegrated, practically all the proto-pectin present can be dissolved in cold distilled water. The process of solution is very slow, however, and Carré<sup>63</sup>, who disputes the validity of Tutin's conclusions, ascribes the gradual solution of the proto-pectin observed by Tutin to its slow conversion to soluble pectin through hydrolysis.

The constitution of the pectin molecule is far from clear. Von Fellenberg<sup>58</sup>, Ehrlich<sup>64</sup>, and others have obtained a pentosan, an araban, from proto-pectin. This araban, according to Ehrlich, does not form part of the pectin molecule, which he regards as the calcium magnesium salt of pectic acid, an anhydro-arabino-galacto-methoxy-tetra-galacturonic acid with which one group of araban is admixed or loosely held. The analysis of cytopectic acid by Schryver and his collaborators shows carbon, hydrogen, and oxygen in proportions indicating the presence in the molecule of two hexose groups and one pentose group. These authors express the view that cytopectic acid is present in plant tissues in chemical combination with cellulose, but do not support this statement experimentally.

In an interesting study of the changes occurring in pectin during retting, Correns<sup>65</sup> comes to the conclusion that protopectin is a fully saturated methoxyl ester of pectic acid. As the retting progresses this ester is saponified and the liberated carboxyl groups are immediately saturated with calcium.

On hydrolysis with dilute acids, the plant gums yield decomposition products which in many respects resemble those obtained from pectin. Thus arabinose, a pentose, is constantly found, while galactose, a hexose, is produced in even larger quantities. In addition, compounds of an acid nature are obtained.

Some gums, such as gum arabic, readily form colloidal solutions with water, while others, such as tragacanth, absorb water and swell without going into solution. In the case of the latter type it is necessary to add an alkali, such as sodium hydroxide, to render them soluble. The gums which are soluble in water usually show a slightly acid reaction. They may be precipitated from their solution by alcohol. Schweizer's reagent does not dissolve gums to any appreciable extent and they give no blue coloration with iodine.

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## CHAPTER II

### IMPORTANCE OF MICROBIOLOGICAL ASPECT

It may be of interest to consider the problem of the disposal of the surplus vegetation of the world, and for this purpose to visualize it not in terms of wood or desiccated leaves, but as units of the elements of carbon, oxygen, and hydrogen, of which all vegetation is primarily composed. From these elements, in the form of carbon dioxide and water, the living plant cell builds up the bulk of all vegetable tissues, and it is to carbon dioxide and water that the dead vegetation must again be converted if the balance of nature is to be maintained, and life to be protected against extinction through the accumulation of vegetable debris. The dead vegetation of the world might, of course, be converted into carbon dioxide and water by burning it, but its accumulated energy can be turned to better account and is, in fact, being far more economically utilized in the natural processes of decay, which, like burning, result in its complete combustion.

To take the case of cellulose itself, under natural conditions it breaks down into intermediate soluble compounds still of a carbohydrate nature, which in turn may be converted into organic acids such as acetic and butyric acids. The intermediate decomposition products may assist in the fixation of atmospheric nitrogen and in the accumulation of nitrogen in the soil in a form in which it can be utilized by the higher plants for the synthesis of proteins. In other cases cellulosic materials in the form of lignocellulose may be converted into compounds—the humic substances, which in addition to other valuable properties possess the faculty of retaining moisture tenaciously.

Empirically these facts had been established by the earliest

agriculturists, and they are embodied in the advice given by Virgil to the Roman farmers in the first book of his *Georgics*. But throughout the Middle Ages and up to the last decades of the past century, so long as the belief in spontaneous generation still held sway even among the greatest men of science, a conception of the agencies responsible for the decomposition of the dead vegetation remained obscure. That nature possesses, in fermentation and putrefaction, powerful means for the removal of dead animal and vegetable debris appears first to have been perceived by Reiset<sup>1</sup>. In 1856, in a communication to the Academy of France, he suggested that a study of the gases evolved during the rotting of a manure heap might throw light on the reactions by which nature disposes of dead organic matter.

Reiset's suggestions seem to have been taken up by several investigators during the following two decades, a period which witnessed the final overthrow of the theory of spontaneous generation and saw the dawn of a new and brilliant era for the biological sciences, owing primarily to the researches of Louis Pasteur. As one of the investigators following Reiset's lead, Gayon<sup>2</sup> in 1884 established that there are two different types of fermentation going on in the manure heap, one of which proceeds in the presence, and the other in the absence of air (oxygen). With access of air, carbon dioxide is stated to be the only gas evolved. During the aerobic fermentation a considerable rise in the temperature of the heap occurs, temperatures as high as 72° C. being reached. In the absence of air the heap gives off methane, and its temperature remains more or less stationary. Gayon also states that he succeeded in isolating the agent of this methane fermentation in the shape of a small micro-organism which he found to be capable of fermenting cellulose.

During the same year, and slightly anticipating Gayon, Dehérain<sup>3</sup> gave an account of his investigations into the reactions taking place in the manure heap. These investigations convinced him that two different ferments (micro-organisms) were active in the anaerobic decomposition of the heap, and that these ferments were introduced into the heap

with faecal matter from the animals concerned. He also noted that the temperature of the heap rises only in the presence of oxygen, but did not attribute this rise to the action of micro-organisms. Four years later he reported <sup>4</sup> that a large proportion of the cellulose, as well as of the vasculose (lignocellulose) of the heap, became decomposed as a result of the fermentations, while the nitrogen compounds were converted into ammonia and free nitrogen, the latter escaping into the air.

These observations on the breakdown of cellulose and its associated substances were anticipated by others, one of which dates from the year 1850. In this year Mitscherlich <sup>5</sup> reported that a ferment \* may be prepared by steeping potato slices in water kept at a reasonably high temperature, about 30° C. This ferment is capable of disintegrating further potato slices immersed in it, in such a way as to dissolve the walls of the individual cells and set free the starch granules contained in the cells. This cellulose-dissolving action, according to Mitscherlich, was not due to the presence of fungi in the ferment, but possibly to a *vibrio*, a rod-shaped micro-organism, of which he observed great numbers in the ferment.

During an attempt to isolate the laticiferous cells of certain plants, Trécul <sup>6</sup>, in 1865, observed and investigated micro-organisms which we now know to have been similar to, if not identical with, the vibrio of Mitscherlich. In the liquid in which Trécul digested his latex-carrying tissues he observed three types of microscopic rod-shaped bodies which he termed *Amylobacter*, *Clostridium*, and *Urocephalum* respectively, and which he regarded as having arisen in the liquids through spontaneous generation. It was not until about twelve years later that the real nature of these bodies was elucidated by van Tieghem <sup>7</sup>, who showed them to be stages in the life-cycle of a rod-shaped micro-organism which he termed *Amylobacter*, and which he later showed was responsible for the decomposition of the cellulose of the cell walls of potatoes, and therefore identical with the vibrio observed by Mitscherlich. Van Tieghem also saw in *Amylobacter* the active agent of the

\* Mitscherlich evidently meant a culture of micro-organisms.

retting of the flax stem, and he emphasized that, though it is to be regarded as a cellulose-decomposing type, it does not attack the bast fibres of the flax plant. In spite of this contradiction, which cannot be regarded as surprising, in view of the state of the chemistry of cellulose at that time, *Amylobacter*, for many years after, was regarded as one of the most important micro-organisms in existence for the decomposition of cellulose.

A third line of research into the natural decomposition of cellulose and its associated substances was initiated in 1875 by Popoff<sup>8</sup>, who investigated the causes of the natural evolution of methane in stagnant ponds. He found that methane is produced from cellulose and its related substances, such as gums, deposited at the bottom of ponds, and that a methane fermentation could be artificially started if a flask filled with water and substances containing cellulose were inoculated with mud. The methane evolved during the resulting fermentation was sometimes found to be mixed with small amounts of hydrogen. In 1883 Hoppe-Seyler<sup>9</sup> reported on his investigations in the same field. He was the first to use Swedish filter paper, a resistant type of cellulose, as a source of this substance in his experiments, and from these investigations dates a proper definition of the term 'cellulose fermentation'. Until then, any of the component parts of the vegetable cell wall, whether cellulose proper, hemicelluloses, or pectin, had been regarded as cellulose, a state of affairs not conducive to the elucidation of the rather complex reactions involved in the natural decay of vegetable matter. Hoppe-Seyler's investigations, which will be discussed in some of the subsequent chapters, confirmed that hydrogen may be present with the methane given off during the fermentation of cellulose.

Two further lines of research have still to be mentioned by which the study of the natural decomposition of cellulose and its associated substances was taken in hand. In 1882 Tappeiner<sup>10</sup> gave an account of his researches into the disappearance of cellulose in the intestine of herbivorous animals. These researches led him to the conclusion that the statements, sometimes found in the literature, that the mucous membranes



of the intestines of herbivorous animals produce a cellulose-resolving enzyme, are erroneous and that the resolution of the cellulose in this, as in other cases investigated, is the work of micro-organisms. He also pointed out that cellulose may be decomposed through two different fermentations, in one of which, the methane fermentation, the cellulose yields methane, while in the other, the hydrogen fermentation, it is largely converted into hydrogen. Whether these two fermentations are caused by the same ferment (micro-organism) under varying conditions of growth, or whether two different ferments exist, Tappeiner was unable to decide. Both fermentations may proceed at the same time, a fact which explains why methane naturally or artificially produced from cellulose often contains hydrogen mixed with it.

A fifth line of research into the natural decay of cellulosic materials was initiated in 1886 by de Bary<sup>11</sup>, who reported that he had collected an extract from the mycelium of a fungus, a *Botrytis* species, with which he could dissolve the cell wall of plants. His observations were confirmed by Marshall Ward<sup>12</sup> in 1888, and more convincingly by Behrens<sup>13</sup> in 1898. Since the date of Behrens's investigations, which were carried out with Swedish filter paper as a source of cellulose, doubt no longer exists as to the property of fungi of decomposing cellulose, and an insight has been gained into the important role performed by fungi in the natural disposal of surplus vegetable matter.

Thus, in five different directions the question of the natural disposal of surplus vegetable matter was followed up during the last decades of the past century, and the exploration of each of these lines of investigation has proved essential for an appreciation of the immense importance of micro-organisms in maintaining the equilibrium of the carbon supply of nature.

Before proceeding to give a detailed account of the various micro-organisms which are active in the decomposition of vegetable tissues, the methods by which they are capable of acting remain to be indicated. It was mentioned in Chapter I that the decomposition of cellulose, hemicelluloses, pectin, and gums by mineral acids resulted in the formation of monoses,

either hexoses or pentoses. These reactions are collectively called hydrolysis, because the elements of water are introduced into the molecule of the hydrolysed substances. An hydrolysis resulting in the formation of monoses is also generally assumed to be the first step in the action of micro-organisms on cellulose and its associated substances, but whereas hydrolysis by chemical means requires the expenditure of considerable energy in the form of heat, as well as the presence of an acid, microbiological hydrolysis takes place at ordinary temperatures and at what is, practically speaking, a neutral reaction. The apparent facility with which micro-organisms carry out the hydrolysis of cellulose and the other substances with which it occurs in nature is due to the production by the organism of enzymes. The cellulose-dissolving enzyme which was first described by de Bary<sup>11</sup> is known to-day under the name cellulase or cytase. Its action was studied in some detail by von Euler<sup>14</sup>, who obtained it from the hyphae of the dry-rot fungus *Merulius lacrymans*. He treated the mycelium of this fungus with water, and obtained an extract containing the enzyme. When this was allowed to act on cellulose dextrans the enzyme produced from them reducing sugars, the nature of which, however, was not determined by von Euler. The cellulose dextrans used by him were prepared from filter paper by dissolving it in 70 per cent. sulphuric acid, removal of the sulphuric acid radicals of the cellulose sulphonyl-esters by the addition of barium hydroxide, and dialysis of the filtrate to eliminate any reducing substances present in the filtrate. Evidence of the nature of the reducing sugars formed by cellulase from cellulose was obtained by Pringsheim<sup>15</sup>. This evidence will be discussed in greater detail in Chapter VIII. Here it suffices to mention that cellobiose was obtained by Pringsheim as a soluble decomposition product of cellulose, and that this in turn broke down and yielded glucose.

The first observations made on a *pectin*-hydrolysing enzyme were made by Bourquelot and Hérissé<sup>16</sup> in 1898. This enzyme pectinase was obtained from malt by extraction with water. How far this is identical with the pectin-dissolving enzyme pectinase described by Beijerinck and

van Delden<sup>17</sup> is not clear. According to the latter investigators pectosinase is the enzyme by which the retting bacteria, the *Amylobacter* of Trécul and van Tieghem, dissolve the middle lamellae of the cells of the flax stem. By the action of this enzyme pectin is hydrolysed to arabinose and galactose, which are the same carbohydrates as those obtained from pectin by chemical hydrolysis. Since a large number of bacteria, as well as of fungi, are able to resolve the middle lamellae of plant tissues, it is probable that pectosinase is frequently produced by micro-organisms.

That micro-organisms hydrolyse *hemicelluloses* by means of enzymes is reported by Sawamura<sup>18</sup> in the case of mannans, galactans, and arabans, and by Gran<sup>19</sup> in the case of gelose, the carbohydrate present in agar-agar. The decomposition products as far as determined in these cases are identical with those yielded by the same hemicelluloses through chemical hydrolysis. The same undoubtedly holds good also for the microbiological decomposition of those hemicelluloses which have not yet been investigated.

In the decomposition of *lignocellulose* (wood) by certain fungi it is often observed (von Tubeuf<sup>20</sup>) that the cellulose remains more or less undecomposed. In such cases the lignin has probably been removed by a specific enzyme of which little, however, is so far known.

A further investigation of all these enzymes responsible for the hydrolysis of cellulose and its associated substances would be of considerable interest. To most people these enzymes are little more than names, and no definite opinion can be expressed at present as to the extent of their relationship. That a certain difference exists is unquestionable, since the pectosinase of the *Amylobacter* group, for example, is unable to hydrolyse agar-agar. On the other hand, a relationship between, for instance, cellulase and pectosinase is suggested by the fact that the enzyme produced by the cellulose-decomposing bacteria investigated by Omelianski<sup>21</sup> has been found capable of dissolving both the pectin and the cellulose of the flax stem.

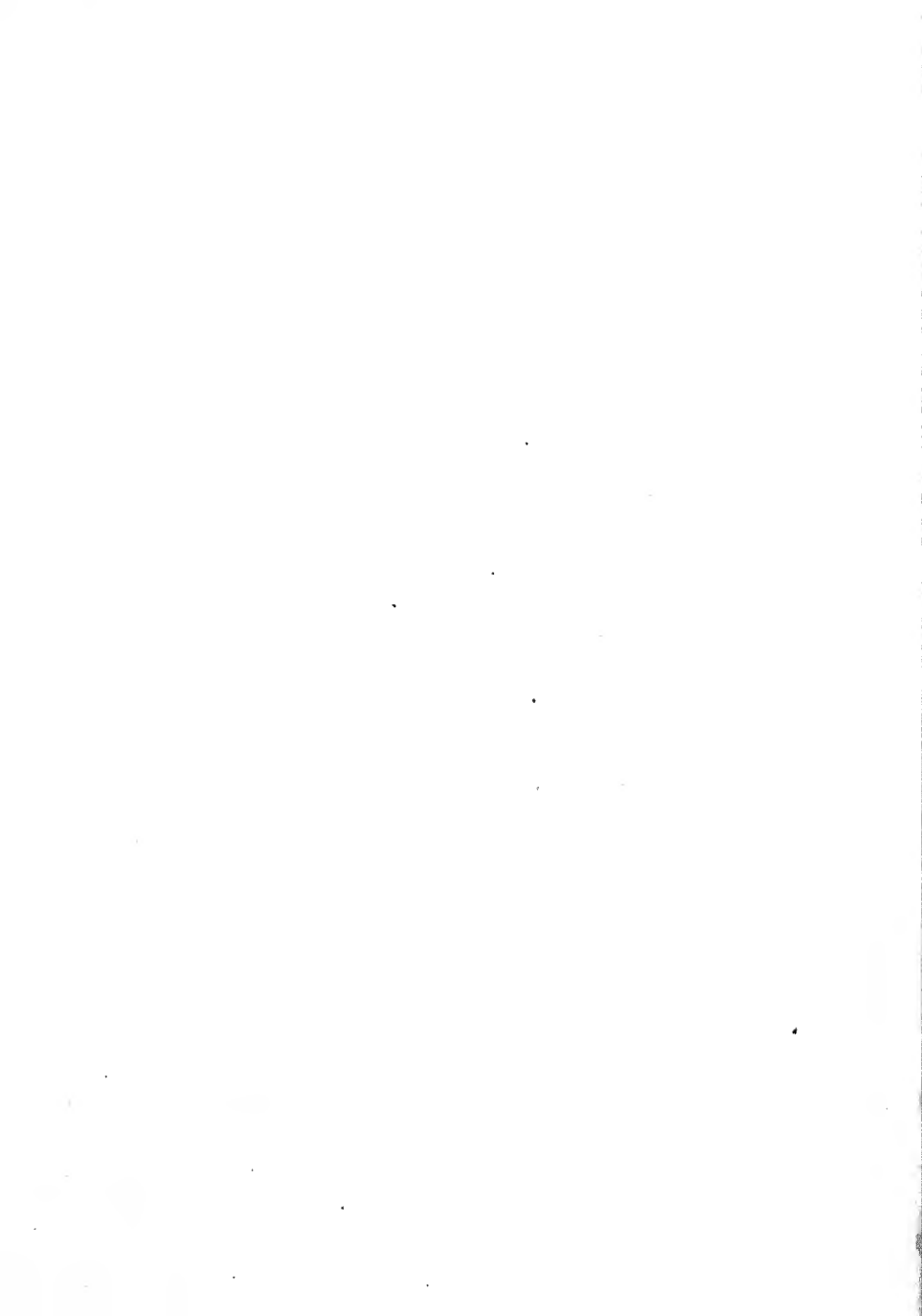
An alternative, of course, would be to assume the presence in the Omelianski bacteria, as well as in many other cellulose-

decomposing micro-organisms, of both a cellulase and a pectinase. In either case, however, existing conception rests on the unsafe foundation of mere assumption, a fact which emphasizes the need for further investigations.

Cellulose, hemicelluloses, pectin, and gums, once converted into soluble carbohydrates, become a most valuable source of energy, not only for the micro-organisms responsible for their hydrolysis, but also for a very large number of other types unable to attack the mother substances. The natural decomposition of vegetable debris, therefore, results in a great complexity of reactions, some of which will be discussed in greater detail in the following chapters.

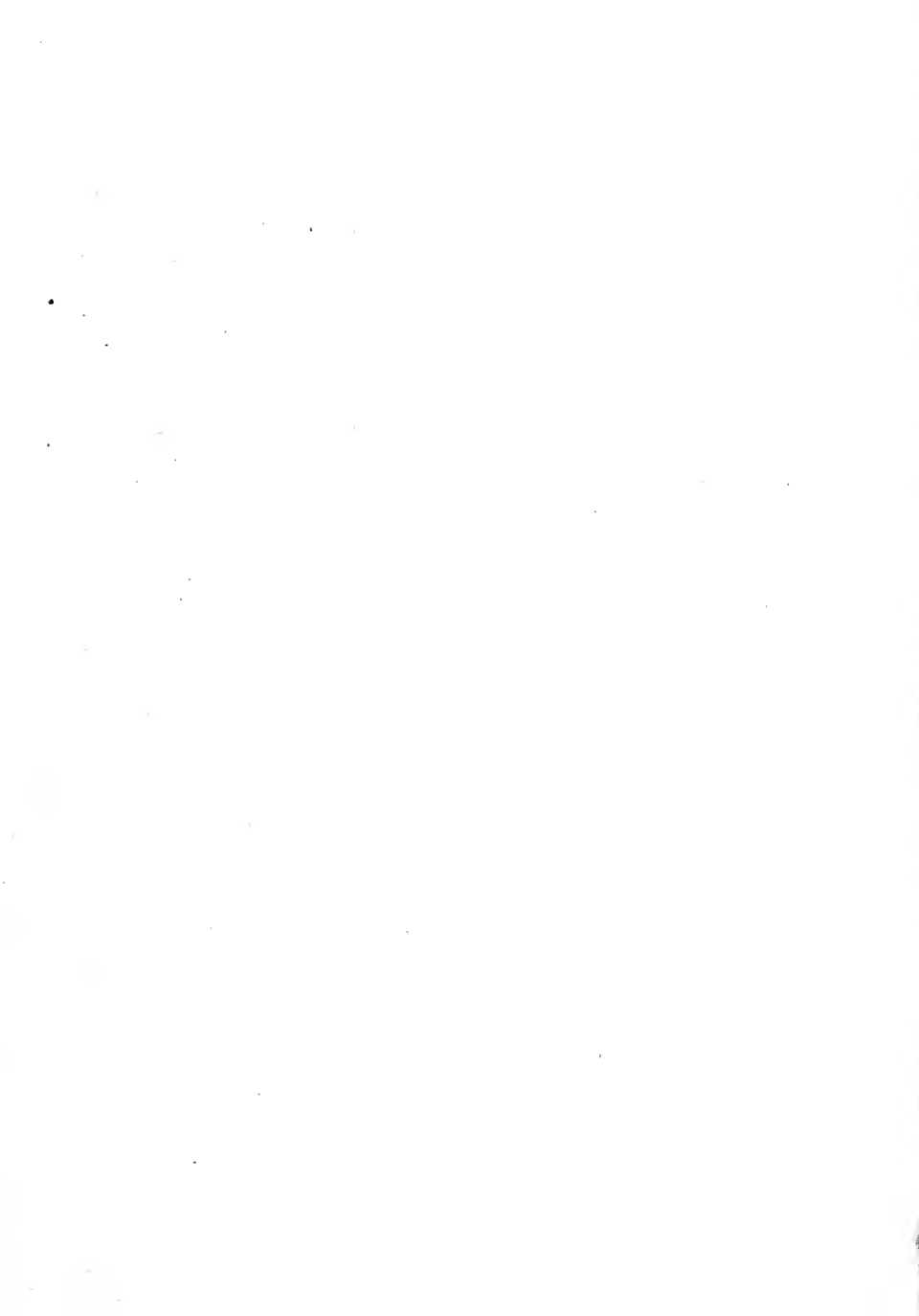
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PART TWO

THE TYPES OF MICRO-ORGANISMS ASSOCIATED  
WITH THE DECOMPOSITION OF CELLULOSE  
HEMICELLULOSES, PECTIN, AND GUMS



### CHAPTER III

## THE SCHIZOMYCETES

IN considering the micro-organisms responsible for the natural decay of vegetable debris through the destruction of cellulose, hemicelluloses, pectin, or gums, a summary of the systematic grouping will first be given. The first class to be mentioned in this connexion is that of the myxomycetes, which has been included here because these organisms are frequently met with on rotting wood, manure, and other decaying vegetable matter. It is extremely problematic, however, whether the myxomycetes take any part in the decomposition of cellulose and its associated substances. Existing knowledge of their physiology rather indicates that they are parasites living on the fungi and bacteria responsible for the natural decay of vegetable debris, and not saprophytes which decompose decaying plant tissues. For the purpose of this volume a detailed discussion of the myxomycetes may therefore be omitted. Readers interested in this group are referred to existing botanical text-books, and to Lister's *Guide to the British Mycetozoa exhibited in the Department of Botany, British Museum (Natural History)*, 3rd edition.

Proceeding from the less to the more complicated forms, the next class of micro-organisms found on decaying vegetation is that of the schizomycetes. That this class is active in the natural decay of plant tissues has been definitely established, and a detailed discussion of the schizomycetes concerned will therefore be given.



Following the usual botanical grouping the next class to be considered are the fungi, or eumycetes. Here almost every order and family contains representatives which decompose either cellulose (including lignocellulose), hemicelluloses, pectin, or gums, and directly or indirectly all fungi, with few exceptions, take a part in the natural decomposition of the world's vegetation, either as parasites or as saprophytes. In view of the enormous number of existing fungi, a detailed description of all the species of this class cannot be given. The account will be limited to those saprophytic genera which have been recorded by investigators as being associated with the breakdown of cellulose, hemicelluloses, pectin, and gums.

That the class following the fungi, the algae, may have a bearing on the natural decay of vegetable matter, through the decomposition of cellulose or its associated compounds, has not been overlooked. With our present knowledge of the physiology of this group it is hardly justifiable, however, to include the organisms of this class in the discussion which follows. The account, therefore, remains limited to the schizomycetes, including such forms as *Spirochaeta cytophaga* (Hutchinson and Clayton<sup>1</sup>), and *Microspira agar-liquefaciens* (Gray and Chalmers<sup>2</sup>), of which at least the former might be regarded by some writers as a protozoon, and to the eumycetes, the latter with the reservations made above.

In the usual subdivision of the schizomycetes, the *Actinomycetaceae*, or ray fungi, are included as a family in the order of the *Eubacteriales*. This classification will not be followed here, however, as recent researches by Ørskov<sup>3</sup> have made it more probable than ever that the actinomycetes do not belong to the schizomycetes. Since their properties also differ in many respects from those of the eumycetes, it has been thought advisable to treat the ray fungi as a separate class under the name of the actinomycetes. The micro-organisms to be considered in the following pages therefore, comprise three classes, the schizomycetes, the actinomycetes, and the eumycetes.

## THE SCHIZOMYCETES

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The schizomycetes are divided into two orders, the *Eubacteriales* and the *Myxobacteriales*, of which the former is by far the more important.

### ORDER I. The Eubacteriales.

Omitting the actinomycetes, the *Eubacteriales* may be divided into five families: (1) the *Coccaceae*; (2) the *Bacteriaceae*; (3) the *Spirillaceae*; (4) the *Phycobacteriaceae* (*Chlamydobacteriaceae*); and (5) the *Thiobacteriaceae* (*Beggiatoaceae*). Of these, the two last-named families may be excluded from the discussion as they obtain the energy necessary for their life functions by the oxidation of ferrous salts and of sulphur respectively. It is with the first three families, therefore, that the following account will deal. No attempt has been made to arrange the various species described in their appropriate families, since such a grouping would give but a vague idea of the complexity of the process of natural decomposition of vegetable debris. In order to focus attention on the manifold reactions by which the natural decay of cellulose, hemicelluloses, pectin, and gums is accomplished by the bacteria, they have been subdivided on a physiological basis. In the first place, the organisms have been divided into two main groups, the mesophilic forms, active up to a temperature of 45° C., and the thermophilic forms, which develop at temperatures up to 70° C. and have an optimum temperature for growth at 50 to 60° C. Each of these groups has been subdivided into three further groups, the obligatory aerobes, the facultative anaerobes, and the obligatory anaerobes. The two latter have each been subdivided again into two groups, of which one contains the forms which decompose the vegetable debris with the evolution of free nitrogen from nitrates or nitrites, and the other the forms which carry out the decomposition of the carbohydrates without denitrification.\*

The obligatory aerobes, as well as the non-denitrifying facultative and obligatory anaerobes, have been divided into

\* By denitrification is here meant only such reduction of nitrates and other nitrogen compounds as results in the evolution of free nitrogen.

sub-groups according to their ability to carry out the decomposition of the carbohydrates in question simultaneously with the fixation of atmospheric nitrogen, or in the absence of nitrogen fixation.

The above grouping may be summarized as set out in the accompanying table :

## EUBACTERIALES

### A. THE MESOPHILIC BACTERIA.

- |                           |   |  |
|---------------------------|---|--|
| (a) Obligatory aerobes    | $\left\{ \begin{array}{l} 1. \text{ Nitrogen-fixing types} \\ 2. \text{ Types which do not fix atmospheric nitrogen} \end{array} \right.$   |  |
| (b) Facultative anaerobes | $\left\{ \begin{array}{l} 1. \text{ Denitrifying types} \\ 2. \text{ Types which do not liberate atmospheric nitrogen} \end{array} \right.$ | $\left\{ \begin{array}{l} \alpha. \text{ Nitrogen-fixing types} \\ \beta. \text{ Types which do not fix atmospheric nitrogen} \end{array} \right.$ |
| (c) Obligatory anaerobes  | $\left\{ \begin{array}{l} 1. \text{ Denitrifying types} \\ 2. \text{ Types which do not liberate atmospheric nitrogen} \end{array} \right.$ | $\left\{ \begin{array}{l} \alpha. \text{ Nitrogen-fixing types} \\ \beta. \text{ Types which do not fix atmospheric nitrogen} \end{array} \right.$ |

### B. THE THERMOPHILIC BACTERIA.

- |                           |   |  |
|---------------------------|---|--|
| (a) Obligatory aerobes    | $\left\{ \begin{array}{l} 1. \text{ Nitrogen-fixing types} \\ 2. \text{ Types which do not fix atmospheric nitrogen} \end{array} \right.$   |  |
| (b) Facultative anaerobes | $\left\{ \begin{array}{l} 1. \text{ Denitrifying types} \\ 2. \text{ Types which do not liberate atmospheric nitrogen} \end{array} \right.$ | $\left\{ \begin{array}{l} \alpha. \text{ Nitrogen-fixing types} \\ \beta. \text{ Types which do not fix atmospheric nitrogen} \end{array} \right.$ |
| (c) Obligatory anaerobes  | $\left\{ \begin{array}{l} 1. \text{ Denitrifying types} \\ 2. \text{ Types which do not liberate atmospheric nitrogen} \end{array} \right.$ | $\left\{ \begin{array}{l} \alpha. \text{ Nitrogen-fixing types} \\ \beta. \text{ Types which do not fix atmospheric nitrogen} \end{array} \right.$ |

To complete this classification it should be added that, theoretically speaking, each of the sixteen groups should include representatives which utilize either gums, pectin, hemicelluloses, or cellulose as a source of carbohydrate, and others which decompose more than one or possibly all of the above substances indiscriminately.

It will thus be seen that the activity of the *Eubacteriales* may be extraordinarily varied. The grouping suggested emphasizes one of the main difficulties which has to be encountered in the study of the natural decay of vegetable matter by these micro-organisms. Another of these difficulties,

that of isolating the responsible forms in pure culture, is still a very real one, and is the cause of the lack of existing reliable information regarding many of the micro-organisms in these groups.

Though the principle was adopted, in compiling a description of the micro-organisms discussed in this chapter, of not describing types which had not been studied in pure culture, this decision had to be overruled in many instances, particularly in the case of the bacteria, where as often as not pure cultures are still unknown.

#### THE GUM-DECOMPOSING BACTERIA.

Bacteria which utilize gums as a source of carbohydrates are but scantily represented in existing literature. Only three available papers deal, quite superficially, with these micro-organisms.

In the first of these, Stocks<sup>4</sup> reports on the occurrence of black patches, containing bacterial spores, in tragasol gum. When such discoloured gum is left in a reasonably warm place and in the presence of water, several types of bacteria develop, the predominating type being a spore-forming rod with terminal spores. A non-spore-forming rod and a diplococcus may also be observed. Attempts were made to isolate the three types, but they could not be made to develop on ordinary laboratory media. One or all of them decompose the gum with the production of acid. This acid dissolves particles of iron from the machinery used in the preparation of the gum, and the soluble iron salts thus formed interact with the tannins of the gum, producing the black patches referred to.

Recently Greig Smith<sup>5</sup> has reported that a certain thermophilic micro-organism obtained from fermenting tan bark, but of which no description is given, can ferment the gum exuded by species of *Acacia*.

Voskressensky<sup>6</sup>, who studied the digestibility of cherry-tree gum, found that it is slightly attacked by bacteria living in the intestine of edible snails. The nature of these bacteria is not disclosed by him.

That bacteria which utilize gums as their only source of carbohydrate are widely distributed in nature can be proved experimentally by incubating solutions of gum arabic or of gum tragacanth in shallow containers at 20° C., preferably after inoculation of the solutions with soil. After two or three days' incubation the solutions become turbid and increasingly acid, indicating a breakdown of the gums. On examination the solutions are found to contain both spore-forming and non-spore-forming bacteria, the latter often resembling the types described under the name of *Bact. herbicola* *a aureum*.

Similarly, when the gum solutions are kept in deeper containers, both facultative and obligatory anaerobes are found to develop, including both denitrifying types and forms which do not liberate atmospheric nitrogen. Of the nature of these bacteria nothing is known at present, and a detailed investigation of this group has still to be undertaken. Hitherto, the natural decomposition of gums has generally been attributed to the cellulose-decomposing bacteria. Thus Popoff<sup>7</sup> reported as early as 1875 that an organized ferment existed in mud and sewage deposits which converted both gums and cellulose into methane. Omelianski<sup>8</sup>, in his classical studies of the fermentation of cellulose, found that gum arabic was a better source of carbohydrate for his cellulose decomposers than the cellulose itself, and remarks that the addition of this gum to a cellulose fermentation, which had become stagnant, revived the evolution of gas.

#### THE PECTIN-DECOMPOSING BACTERIA.

##### (A) The mesophilic, pectin-decomposing bacteria.

(a) **The mesophilic, aerobic forms.** The misconception of the real nature of pectin in the early days of the history of cellulose research, and the considerable industrial importance of the removal of pectin from plants yielding textile fibres, have both been important factors in the elucidation of this group of bacteria.

The mesophilic types, developing at temperatures up to 45° C., include typical aerobic forms related to, or identical with, *Bac. mesentericus*, (Ehrenberg) Cohn, and *Bac. vulgatus*,

(Flügge) Migula, which, in 1902, were shown by van Hall<sup>9</sup> to cause a white rot of potatoes and of Jerusalem artichoke tubers. That the *Bac. mesentericus* group is capable of dissolving the middle lamellae of the flax stem has been confirmed by Beijerinck and van Delden<sup>10</sup>.

To the aerobic pectin decomposers also belong some of the many plant-pathogenic bacteria, of which the first was studied by Heinz<sup>11</sup> in 1889 under the name of *Bact. hyacinthi septicum*. These plant-pathogenic forms are short non-spore-forming rods approximately  $0.8\ \mu$  broad by 2 to  $3\ \mu$  long. In most of their biochemical reactions they resemble the *Bact. fluorescens* group or those *Bact. coli* forms which are regularly found in grass and hay. They gain access to their host through wounds or through the stomata of the epidermis, and dissolve the middle lamellae of the parenchymatous tissues. On microscopical examination of the host the bacteria are found in large numbers between the cells. They do not, however, penetrate into the cell, which shows that they are incapable of decomposing the cellulose layer of the cell wall. For a detailed description of this important group, which has been carefully investigated by Smith<sup>12</sup> and others, reference should be made to text-books on plant pathology.

Many authorities also include in the aerobic pectin decomposers the two important spore-forming rods *Bac. asteroides*, Meyer<sup>13</sup>, and *Bac. comesii*, Rossi<sup>14</sup>, two closely related species, of which the latter has recently been utilized industrially in Italy, France, and Germany for the retting of flax and hemp. Beijerinck and den Dooren de Jong<sup>15</sup> regard these spore-forming rods, as well as some other pectin-dissolving forms, as facultative anaerobes, since they are capable of living anaerobically in the presence of suitable carbohydrates. These authors group them in one species under the name of *Bac. polymyxa*.

*Bac. asteroides*, (Meyer) Migula, is a motile rod, measuring 1 to  $1.2\ \mu$  by 3 to  $6\ \mu$ , with large oval spores formed eccentrically or terminally, and showing marked longitudinal ridges, which make them appear star-shaped in optical cross-section. The species liquefies gelatine and forms small, slightly raised, transparent, yellowish, and concentric colonies on agar. In broth it produces uniform turbidity, and on potato slices it forms a thick, slimy

coating which discolours the potato yellowish-brown. It ferments dextrose with the evolution of gas and the production of acid. It forms traces of sulphuretted hydrogen. Starch is hydrolysed and indol not produced. *Bac. asterosporus* is stated to fix atmospheric nitrogen, and might, therefore, be placed in a group by itself.

*Bac. macerans*, Schardinger<sup>16</sup>, which was found as an accidental infection in fractionally sterilized potato mash, is stated by its discoverer to be related to *Bac. asterosporus*. It has also been isolated from a retting pond in Langenfeld.

*Bac. comesii*, Rossi<sup>14</sup>, forms oval spores which have no markings and which do not cause a swelling of the mother cell in forming. In its general characteristics it resembles *Bac. mesentericus*.

(b) **The mesophilic, facultative anaerobic forms.** To this group belong the bulk of the above-mentioned plant-pathogenic micro-organisms of the *Bact. fluorescens* type (Brooks, Nain, and Rhodes<sup>17</sup>), and the following spore-forming bacteria: *Bac. leguminiperdus*, described by von Oven<sup>18</sup> as the cause of a destructive disease of young pea pods; *Bac. malakofaciens*, isolated by von Wahl<sup>19</sup> from tinned carrots; *Bac. violaceus acetonicus*, isolated by Bréaudat<sup>20</sup> from drinking-water in Saigon; *Bac. oleae*, obtained by Schiff-Giorgini<sup>21</sup> from tubercles of the olive tree; and probably *Bac. macerans*, isolated by Schardinger and referred to above.

*Bac. malakofaciens*, von Wahl, measures  $0.7\ \mu$  by  $2.7\ \mu$ , is motile, and forms spores measuring  $0.7$  to  $0.8\ \mu$  by  $1\ \mu$ . Two spores are frequently present in each cell. The vegetative cell is slightly motile. In its general characteristics *Bac. malakofaciens* is stated to resemble *Bac. asterosporus*.

*Bac. violaceus acetonicus*, Bréaudat, measures  $1\ \mu$  by  $3\ \mu$ , is motile, and forms spherical central spores measuring  $0.8\ \mu$  to  $1\ \mu$ . It produces purple colonies on agar, when grown aerobically in the presence of peptone. From various carbohydrates it produces acetone and ethyl alcohol in addition to volatile acids.

In addition to the above, five facultative anaerobes capable of decomposing pectin were described by Kayser and Delaval<sup>22</sup> in 1920.

They are all short, non-spore-forming, motile rods of the 'grass coli' type, measuring  $0.5$  to  $0.6\ \mu$  by  $1.8$  to  $2.0\ \mu$ . Their further characteristics, so far as information is available from Kayser and Delaval's account, are as follows:

*Strain I*, isolated from a flax infusion, forms yellowish, transparent, and slightly slimy colonies on the ordinary laboratory media, and peptonizes milk.

*Strain II*, obtained from a hemp maceration, forms similar colonies, but produces a small quantity of gas when fermenting sugars. Milk is curdled without peptonization, indicating the production of acid on the decomposition of lactose.

*Strain III*, isolated from a maceration of ramie, shows similar colonies to those of *Strain II*, and coagulates and peptonizes milk.

*Strain IV*, found in a jute maceration, appears to give the same reactions as *Strain II*.

*Strain V*, from a hemp maceration, forms greyish colonies on agar which are transparent and somewhat slimy, like those of the other types. Milk is peptonized without being curdled.

All the strains ferment monoses and disaccharides, the former more readily than the latter. Pectin is decomposed by all of them, and most completely by *Strain II*, which converts 56.7 per cent. of the pentoses contained in the pectin.

A further anaerobic pectin decomposer, isolated by Kayser and Delaval, is described as follows:

The vegetative cells measure 1.3 to 1.7  $\mu$  by 5.1 to 8.5  $\mu$ . The spores are round and terminal, and are sometimes formed at both ends of a rod. The rod is motile. Gelatine is liquefied. The colonies on agar are whitish and somewhat slimy. Growth is sparse on potato, but abundant on carrot and beet.

None of the mesophilic, facultative anaerobic types is able to liberate atmospheric nitrogen, and none is known to fix atmospheric nitrogen, with the possible exception of *Bac. malakofaciens*.

A short bacillus was obtained from the intestine of the hen by Distaso<sup>23</sup>, and was described by him under the name *Bacillus cellulosaе desagregans*. As its discoverer himself suggests, it is more likely to be a pectin decomposer than a fermenter of cellulose, since it develops well on peas, and limits its action on cellulose (filter paper) to a reduction of the coherence of the mass of fibres.

It is stated to be a short rod with straight ends forming terminal to sub-terminal spores. It liquefies gelatine, has no action on milk, and does not form indol. Glucose is slightly decomposed, while others of the more common sugars are not. Starch, however, is hydrolysed. Its colonies on agar are small, transparent, with smooth edges, and resemble those of pathogenic streptococci.



(c) **The mesophilic, obligatory anaerobic forms.** The obligatory anaerobic pectin decomposers are perhaps the most interesting of all the pectin-fermenting bacteria. They are widely distributed in nature and are found in soils wherever plant tissues decay. At one time they were regarded as the micro-organisms responsible for the natural breakdown of cellulose, and as such they were originally introduced into the microbiological literature in 1850 by Mitscherlich<sup>24</sup>. He found that potato slices, left to rot in water at suitable temperatures, formed an active ferment by means of which the starch of the potato cells could be liberated. A similar 'ferment' was studied by Trécul<sup>25</sup> in 1865, and the rods observed in it were named by him *Amylobacter*, *Urocephalum*, and *Clostridium*, names which have since become firmly established in the technical nomenclature. Fourteen years later van Tieghem<sup>26</sup> undertook a careful study of Trécul's micro-organisms, and found that they were stages in the life-cycle of one single type, which he termed *Bac. amylobacter*. This type he regarded as the typical cellulose fermenter, and found it to be identical with *Vibrio butyrique*, Pasteur<sup>27</sup>. Prazmowski<sup>28</sup>, who investigated *Bac. amylobacter* at the same time, disagreed with van Tieghem in regarding it as a cellulose decomposer. Instead, he described<sup>29</sup> two other types, *Clostridium polymyxa* and *Vibrio rugula*, as the principal cellulose decomposers.

*Clostridium polymyxa* was found to be particularly active in preparations of cooked potato and of lupine seed. From the description given of this type it may safely be regarded as a pectin decomposer pure and simple. It is considered by Beijerinck and den Dooren de Jong<sup>15</sup> to belong to this group and is included by them in their species *Bac. polymyxa*.

*Vibrio rugula*, which is no more a vibrio than Pasteur's *Vibrio butyrique*, forms terminal spores, is motile, and measures up to 8  $\mu$  in length. According to Lehmann and Neumann<sup>30</sup> it resembles *Bac. oedematis maligni* in its characteristics. It should probably be regarded as a pectin decomposer and not, as McBeth and Scales<sup>31</sup> would prefer, as a type very similar to the cellulose decomposers isolated by Omelianski<sup>32</sup>.

During the last decade of the past century a large number of types related to *Bac. amylobacter*, van Tieghem, were isolated from the retting liquor of flax and hemp, and from decaying parenchymatous tissues of roots and tubers such as potatoes. At least one of them, *Clostridium Pasteurianum*, Winogradsky<sup>33</sup>, was definitely shown to be incapable of decomposing cellulose in the form of filter paper. All of these types, whether described as *Clostridium Pasteurianum*, Winogradsky<sup>33</sup>; *Plectridium pectinovorum*, Störmer<sup>34</sup>; or *Granulobacter pectinovorum*, Beijerinck and van Delden<sup>10</sup>, were regarded by Bredemann<sup>35</sup> as one species and grouped by him under the name of *Bac. amylobacter*, A. Meyer and Bredemann. Though this grouping may disregard some of the finer details of the reactions of these forms, such, for instance, as the proportions of alcohols produced by them in the fermentation of carbohydrates, it is substantially correct, and will be adhered to in the following description of the type (see also Fig. 1).

*Bac. amylobacter*, A. Meyer and Bredemann, is a motile rod, measuring 1 to  $1.5\ \mu$  in width and 3 to  $5\ \mu$  in length. It forms large spores which are placed either in the centre of the mother cell, giving it a spindle form (*Clostridium*), or towards one end, when the mother cell becomes club-shaped (*Plectridium*). It usually forms a reserve substance known as amylose, which may be stained blue or deep purple with iodine.

*Bac. amylobacter* ferments pectin as well as many carbohydrates, including starch and pentoses. By this fermentation the carbohydrate molecule is broken down into acetic and butyric acids, with larger or smaller quantities of butyl alcohol, ethyl alcohol, acetone, and traces of other fatty acids. During the fermentation appreciable quantities of hydrogen and carbon dioxide are liberated. Some of the species belonging to *Bac. amylobacter* produce tryptic enzymes and consequently liquefy gelatine, and all are capable of fixing atmospheric nitrogen when grown in media free from, or with only traces of, organic or inorganic nitrogen. Even in the most favourable circumstances the growth of *Bac. amylobacter* on ordinary solid laboratory media is indifferent and special methods are usually required for its isolation.

Störmer<sup>34</sup> recommends a preliminary cultivation in a medium composed of 0.2 to 0.5 per cent. pectin, 0.5 per cent. peptone, 0.1 per cent. potassium di-hydrogen phosphate, and 0.025 per cent. magnesium sulphate, dissolved in water and with calcium carbonate added to neutralize any acid formed during the development of the bacillus. Before each fresh inoculation into this medium the culture is pasteurized to suppress all non-spore-forming types. After four to five sub-cultures in this medium the crude culture of the bacillus may be grown as colonies and isolated in pure culture on a medium prepared from fresh pea seedlings (0.2 per cent.), macerated with water and containing in addition 1 per cent. glucose, 0.25 per cent. asparagin, and 10 per cent. gelatine, the reaction of the medium being adjusted, preferably with malic acid, to a pH of 5.5 to 6.0, at which point blue litmus changes to slight purple.

The writers have tried this method and found it no better than the other media suggested for the isolation of *Bac. amylobacter*, which must continue to rank as one of the most difficult types to isolate in pure culture. A certain amount of success may be obtained when using ordinary wort agar under anaerobic conditions.

With *Bac. amylobacter* may, perhaps, be grouped *Bac. felsineus* isolated by Carbone<sup>35</sup>, on which further information will be given in the description of the various retting processes, and *Pectinobacter amylophilum*, Makrinov<sup>37</sup>, which is claimed by its discoverer to be incapable of fixing atmospheric nitrogen. Otherwise it appears from the description given to resemble *Bac. amylobacter*.

Among the obligatory anaerobic pectin decomposers none is known which liberates atmospheric nitrogen.

#### (B) The thermophilic, pectin-decomposing bacteria.

This group is entirely unexplored and existing literature contains no reference to such forms.

#### THE HEMICELLULOSE-DECOMPOSING BACTERIA.

The information available regarding the decomposition of hemicelluloses by bacteria is scanty, and a subdivision of the few known types on the basis of the system suggested on page 30 would be of little value.

In 1902 Gran<sup>38</sup> described a marine bacterium, *Bact. gelaticum*, which is capable of dissolving gelose, the hemicellulose present in agar-agar. This is a short, mesophilic, and facultatively anaerobic rod, which is actively motile and measures  $0.6\ \mu$  by 2 to  $3\ \mu$ . Gran distinguishes three types of this organism: type  $\alpha$ , var. *genuina*, with transparent pinkish colonies; type  $\beta$ , var. *inergica*, with transparent dirty-white or yellowish colonies; and type  $\gamma$ , var. *bergensis*, with colonies similar in appearance to those of variety  $\beta$ . Of the three types, *Bact. gelaticum*, var. *inergica*, produces the largest amount of gelase and much diastase, *Bact. gelaticum*, var. *bergensis*, produces much diastase, but little gelase, *Bact. gelaticum*, var. *genuina*, produces little of either enzyme.

Gran obtained these organisms from sea water, and recommended the following as the most reliable method of isolating agar-resolving bacteria. A piece of a *Florideae* alga is boiled up in sea water and allowed to cool. To this decoction is added a fragment of a *Florideae* alga, and the liquid is incubated at  $20^{\circ}\text{C}$ . After 24 hours, the gelase-producing bacteria are sufficiently developed to be isolated on an agar medium composed as follows: sodium chloride 3 per cent., di-potassium hydrogen phosphate 0.1 per cent., ammonium chloride 0.1 per cent., and agar sufficient to solidify (i. e. about 1.5 per cent.).

The appearance of the liquefied zone around the agar-resolving colonies which form on this medium is not always very distinct. For this reason Gran recommends the treatment of the medium on which colonies have appeared with a fairly strong solution of potassium iodide. That part of the medium which has remained unaffected by the gelase assumes a purplish-blue colour, while the resolved zones remain colourless.

Two further gelose-decomposing bacteria, both of which are mesophilic and facultatively anaerobic, have been described, one by Panek<sup>39</sup> in 1905 and the other by Biernacki<sup>40</sup> in 1911. They were termed *Bact. betae viscosum* and *Bact. Nenckii* respectively.

*Bacterium betae viscosum*, Panek. When grown on media which are free from sugar it develops as very short rods of from  $0.6$  to  $0.8\ \mu$  by  $1\ \mu$ , with rounded or sometimes pointed ends. They may occur in long or short chains. On stretching, before its division into two daughter cells, the mother cell shows a lighter central zone at the place where division finally occurs. When grown in

media containing sugar; notably saccharose, the cells are longer and not infrequently club-shaped. In or on such media a large amount of mucilage is formed. On ordinary gelatine the colonies do not exceed a diameter of 0.5 mm. They are golden-yellow and finely granulated. On ordinary agar they are a more greyish-yellow. On gelatine containing glucose or saccharose the growth is luxuriant, and after a few days' incubation the circular colonies reach a diameter of from 1 to 2 mms. More characteristic still is the growth on agar containing these sugars, where the originally circular colonies gradually coalesce and form flat to concave mucilaginous colonies of a diameter of from 0.5 to 1.0 cm. The liquefaction of the agar is particularly noticeable when the organism is grown on slopes. Here the medium may gradually become so soft that it sinks to the bottom of the tube. On beet juice gelatine, where the development is as vigorous as, or even more vigorous than, the growth on sugar-containing agar, the formation of mucilage is particularly noticeable, and the colonies may be pulled out in long strands. The optimum temperature for growth lies between 18 and 22° C. At 37° C. very little growth occurs. The organism produces appreciable quantities of acetic and lactic acids as well as dextran and mannite from a number of sugars, particularly from saccharose.

*Bacterium Nencki*, Biernacki, was obtained from a sample of dried Spanish Malaga grapes. It is a short rod measuring 0.8  $\mu$  by 1.25 to 2  $\mu$ . Grown in grape juice, the rods are very short, almost coccoid. On potato the cells may reach a length of 5  $\mu$ . It is facultatively anaerobic and non-motile. In an unstained preparation it shows the presence of a capsule. On gelatine the colonies are yellowish-white and granular. The addition of from 2 to 5 per cent. of glucose to the medium favours the growth, and renders the colonies not only larger but also slightly mucilaginous. The gelatine is not liquefied. On agar plates incubated at 35 to 38° C. the organism grows well, forming greyish-white, mucilaginous, and finely granular colonies reaching 3 mms. in diameter. The addition of glucose and saccharose, as well as of lactic acid, favours the growth. A slight fruity odour is noticeable in such plates. A liquefaction of the agar occurs. Gas is evolved when the organism is grown anaerobically on sugar-containing media. The growth on glycerine potato slices is abundant and creamy. Litmus glucose broth and milk become acidified and have a pleasant odour.

In 1924 Aoi<sup>41</sup> obtained a pure culture of a particularly vigorous agar-decomposing bacterium. He isolated this type from manure prepared from rice straw, and from soil obtained in the neighbourhood of his laboratory, where it occurred in association with cellulose-decomposing bacteria. This organism,

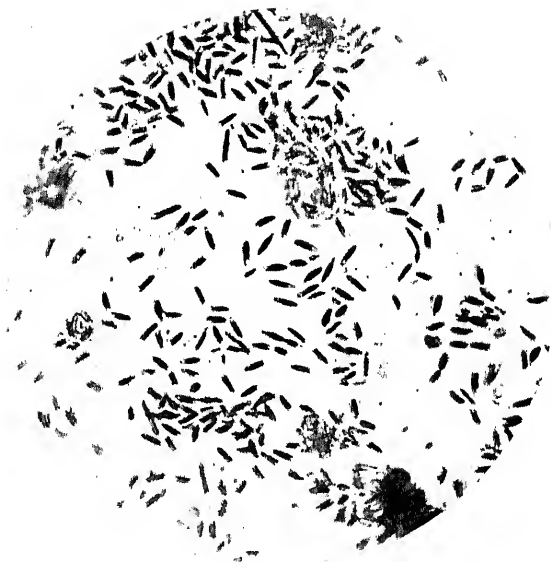


FIG. 1. *Bac. amylobacter*, 24-hours' old culture, stained with iodine and showing the accumulation of amylose in the cells. Magnification  $\times 1,000$ .

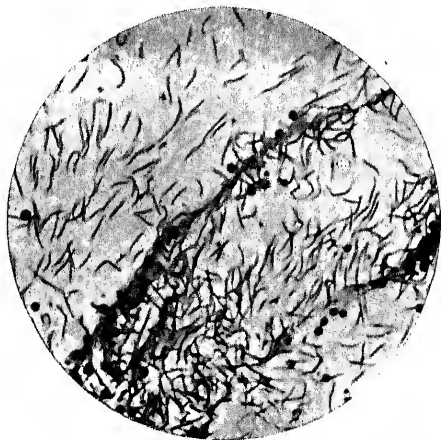


FIG. 2. *Spirochaeta cytophaga*, showing the final stages in the resolution of a cellulose fibre. Both thread form and sporoid are to be seen. At the place marked by + is shown the initial stage of sporoid formation. Magnification  $\times 1,000$ . (From Hutchinson and Clayton's paper, by the courtesy of the *Journal of Agricultural Science*.)



which is unnamed, does not develop on ordinary laboratory media, but grows well on cellulose agar as recommended by Kellerman and his collaborators<sup>42</sup>.

The organism measures  $0.7\ \mu$  by  $2$  to  $3\ \mu$  and is curved, tapering at the ends. Aoi expresses the view that it is a *vibrio*. In old cultures it may reach  $10\ \mu$  or more in length. Stained by Löffler's method the young cells show one polar flagellum. On cellulose agar the colonies become visible after three days' incubation at  $25^{\circ}\text{C}$ . They are white, glistening, and circular. The agar shows infundibuliform liquefaction. On slopes the growth gradually becomes straw-coloured, and the liquefied agar accumulates at the bottom of the tube. It shows marked Fehling-reducing properties.

Among the bacteria which decompose gelose might also be placed the recently described *Microspira agar-liquefaciens*, Gray and Chalmers<sup>2</sup>, of which details will be given in the description of the cellulose-decomposing bacteria.

In addition to these types which decompose the comparatively unimportant hemicellulose of agar-agar, others have been studied which break down the hemicellulose of vegetable ivory. To obtain such types Pringsheim<sup>43</sup> placed shavings of vegetable ivory in water to which ammonium nitrate (50 grammes per litre) had been added as a source of nitrogen, and calcium carbonate as a neutralizing agent to remove any acid formed during the decomposition of the hemicellulose. The microflora which developed on incubation was not studied in detail, but it was established that the vegetable ivory broke down and yielded organic acids.

That the mannan, araban, and galactan in the mucilage produced by *Hydrangea paniculata*, used in Japan for paper sizing purposes, can be decomposed by bacteria was pointed out by Sawamura<sup>44</sup>. The most active organism was found to be *Bac. vulgatus*, (Flügge) Migula, syn. *Bac. graveolens*, Gottheil. *Bact. prodigiosum*, Lehmann and Neumann, also appeared to contain traces of the requisite enzymes.

The information available on the bacterial decomposition of the pentosan xylan, sometimes described as wood gum, is more extensive. The first observations were made by Dupont<sup>45</sup>, who describes a thermotolerant bacillus, *Bac. mesentericus*



*ruber*, which decomposes the xylan present in the straw of the manure heap. In its general characteristics this organism is identical with *Bac. mesentericus*, Flügge, but it develops slowly at ordinary temperatures. At 50° C. its growth is much accelerated and it rapidly forms an abundant wrinkled dirty rose-coloured layer on potatoes.

Another aerobic soil bacillus, *Bac. Ellenbachensis*, Stutzer, was found by Stoklasa<sup>46</sup> to decompose xylan. This type was regarded as synonymous with *Bac. Megatherium* by Stoklasa, but is placed in another genus by Lehmann and Neumann<sup>30</sup>.

A breakdown of xylan in the large intestine of the guinea pig was observed by Seillière<sup>47</sup>, acetic and butyric acids being formed as decomposition products in the proportion of nine parts of the former to one part of the latter. The responsible bacteria in this case may have been related to the pentose-fermenting bacteria studied by Fred, Peterson, and Davenport<sup>48</sup> under the name of *Lactobacillus pentoaceticus*, which are claimed to be able slowly to decompose xylan.

Since *Lactobacillus pentoaceticus* does not form endospores, as might be implied from the name given to it by its discoverers, it will be referred to in this volume as *Bacterium lactipentoaceticum* (see also Peterson and Fred<sup>49</sup>).

It is a rod measuring 0.6 to 0.7  $\mu$  by 1.6 to 3  $\mu$ . Grown on yeast-water agar at 30° C., its smooth uncharacteristic colonies reach a diameter of 1 mm. in three days. On ordinary agar growth is slower. Gelatine is not liquefied. It ferments glucose, galactose, and mannose with the formation of lactic acid, ethyl alcohol, and acetic acid, and with the evolution of carbon dioxide. The acetic acid is probably not a direct fermentation product, but the result of a secondary fermentation of the lactic acid. Xylose is fermented without measurable gas evolution, the same decomposition products being obtained. Milk remains unchanged, and nitrates are not reduced. Nevertheless, methylene blue is reduced, indicating the presence of at least one type of reductase. The optimum temperature for growth lies between 30 and 35° C.; the thermal death-point of the organism between 55 and 60° C.

## THE CELLULOSE-DECOMPOSING BACTERIA.

A. (*a*) Mesophilic aerobic forms.

1. **Nitrogen-fixing types.** No bacteria which oxidize cellulose to obtain energy for the fixation of atmospheric nitrogen have yet been described. Pringsheim<sup>50</sup> has shown, however, that certain free-living nitrogen-fixing micro-organisms can utilize cellobiose, the intermediate decomposition product of cellulose, for this purpose. Pringsheim's further observation, that sufficient cellobiose to satisfy the energy requirements of nitrogen-fixing micro-organisms is formed by cellulose decomposers, is of the greatest interest. It shows that the natural disposal of dead vegetable matter is to be regarded not merely as a removal from the surface of the earth of unnecessary or perhaps even harmful detritus, but also as a natural means of enriching the soil in nitrogen by rendering possible a symbiotic development of these two micro-organisms.

2. **Types which do not fix atmospheric nitrogen.** The first micro-organism belonging to this group was described by van Iterson, jr.<sup>51</sup>, in 1903 under the name of *Bac. ferrugineus*, or *Bact. ferrugineum* by the nomenclature followed in this volume, a rather unfortunate name since it had already been allotted by Rullmann<sup>52</sup> to another rod, isolated from water, which does not decompose cellulose. Van Iterson's organism is stated to be a slender, vigorously motile non-spore-forming rod, the dimensions of which are not given. It is found in the mud of ditches, in garden soil, humus, and on dry leaves.

It may be obtained by placing in a glass dish two pieces of filter paper, between which some powdered ammonium magnesium phosphate is sprinkled, and moistening the paper with a 0.05 per cent. solution of di-potassium hydrogen phosphate. The filter paper is then infected with one of the above substances, and the dishes incubated at about 28° C.

After four to five days' incubation yellowish-brown spots appear on the paper. In these places the paper gradually becomes decomposed and slimy, and on microscopical examination shows several types of micro-organisms, among which

two types predominate, the above-mentioned *Bact. ferrugineum* and a large coccus. In the later stages of the decomposition the remnants of the fibres are enveloped in a mucilaginous mass of the micrococci. Van Iterson ascribes the actual decomposition of the cellulose to *Bact. ferrugineum* and regards the coccus as an organism living in symbiosis with the rod and assisting it in the cellulose decomposition, though unable by itself to break down this carbohydrate.

Recent investigations by Hutchinson and Clayton<sup>1</sup> have thrown fresh light on van Iterson's observations, and it is highly probable that *Bact. ferrugineum* and its accompanying coccus are identical with *Spirochaeta cytophaga*, Hutchinson and Clayton.

This interesting organism, of which a photomicrograph is shown in Fig. 2, was isolated during an investigation of the aerobic cellulose decomposition of Rothamsted soils.

Quantities of 1 gm. of these soils were placed in 300-c.cs. Erlenmeyer flasks, containing 100 c.cs. of a mineral salt solution of the following composition: potassium di-hydrogen phosphate 0.1 per cent., calcium chloride 0.01 per cent., crystalline magnesium sulphate 0.03 per cent., sodium chloride 0.01 per cent., ferric chloride ( $\text{Fe}_2\text{Cl}_6$ ) 0.001 per cent., and sodium nitrate 0.25 per cent.

The hydrogen-ion concentration of the solution was adjusted to about neutral point by the addition of the requisite amount of sodium hydroxide. Before sterilization 1 gm. of filter paper was placed in each flask in such a way as to remain partly above and partly below the surface of the liquid.

After sterilization the flasks with the above medium were inoculated with soil and incubated at 25° C. for from four to six days, after which time the paper became discoloured a yellowish-brown at, or just above, the surface of the liquid. On further incubation the paper lost its consistency and became slimy at the discoloured patches, which were found on microscopical examination to contain numerous slender rods and large cocci. After many unsuccessful attempts at separating the rod and the coccus, Hutchinson and Clayton succeeded in obtaining what appeared to be a pure culture of the rod by adopting the classical dilution method utilized by Lister for the isolation of *Bact. lactis acidii*. From this culture

of the rod, which under the microscope showed absolutely no coccus forms, high dilutions were immediately made and used to inoculate fresh media. The cultures appearing in the fresh media were found, however, after incubation for some days, to contain both rods and cocci. Since the inoculant used could certainly not have contained anything like the number of cocci required to leave even one coccus in the amount of suspension (one-forty millionth of a cubic centimetre) used for starting the second set of cultures in which the coccus reappeared, Hutchinson and Clayton rightly concluded that the coccus is a phase of the life-cycle of the rod. Though the rod, or thread form, reproduces itself by fission, like all other schizomycetes, it shows marked differences from this class of micro-organisms, and Hutchinson and Clayton regard it as approaching more closely to the *Spirochaetaceae*. For this reason they named their cellulose decomposer *Spirochaeta cytophaga*.

In young cultures, *Spirochaeta cytophaga* shows a predominance of rods or threads, measuring 0.3 to 0.4  $\mu$  by about 3  $\mu$ , tapering towards the ends, and frequently curved. Although no flagella can be observed on the thread it nevertheless shows a marked, though slow, movement of a rotatory nature. It possesses perfect flexibility, and therefore often becomes S-, O-, or U-shaped. As a culture becomes older the coccus form, or 'sporoid stage', becomes more frequent. The sporoid measures 1.5  $\mu$  in diameter and is comparatively easily stained, whereas the thread form does not take stains well. Boiling carbol fuchsin is the most satisfactory stain to be applied.

That the sporoid is not to be regarded as a spore in the usual sense of the term is shown by the fact that the sporoid is as readily destroyed by heat as the thread, an exposure to 60° C. for ten minutes being sufficient to destroy both forms. Preceding the sporoid stage the thread form becomes granulated. One such thread with the central granule will be observed in Fig. 2, in the place marked by a cross.

In old cultures, after two or three weeks' cultivation, the bacterial mass surrounding the decayed and decaying fibres appears to consist entirely of the sporoid stage. This probably was the phase observed by van Iterson and described by him

as a 'micrococcus mucilage'. The mucilage produced in such older cultures is suggested by Hutchinson and Clayton as being similar to pectin. This, however, would not appear to be the case, since the mucilage yields no reducing sugars on hydrolysis with mineral acids.

A further point to which Hutchinson and Clayton draw attention in their description of this organism could not be confirmed by experiments carried out by the writers. It is claimed that normal development of the organism takes place in media with an acidity or alkalinity between N/300 HCl and N/160 NaOH, figures which correspond approximately to pH values of 3 and 10 respectively. This, as Hutchinson and Clayton point out, is a somewhat remarkable range. In experiments carried out to confirm this, the writers got good growth after four days' incubation at 28° C. on a strip of filter paper immersed in the mineral salt solution recommended, when the pH was adjusted between 6.94 and 7.01. At a pH of 6.64 the growth was markedly slower, and at a pH of 6.10 no growth took place at all; nor could the organism be induced to develop at a pH of 9.12.

*Spirochaeta cytophaga* is stated to thrive best in the absence of organic nitrogen. Peptone for instance has an inhibitory action on the growth of the organisms in concentrations above 0.25 per cent. As a source of carbohydrates only cellulose is utilized, and others are not only unsuitable but in many cases actually harmful. This is the case with the reducing sugars, dextrose, maltose, and probably cellobiose. *Spirochaeta cytophaga* is therefore to be regarded as an aerobic cellulose decomposer *par excellence*.

In addition to pigment and mucilage, *Spirochaeta cytophaga* produces organic acids from cellulose. The nature of these acids has not been determined. Calculating them as butyric acid, they amounted to about 7 to 9 per cent. of the original cellulose taken.

A type undoubtedly identical with *Spirochaeta cytophaga* was obtained by von Gescher<sup>63</sup> in 1922. The deductions as to the need for symbiotic conditions during cellulose decomposition, which were made by this author from the fact that

both rods and cocci were present in his cultures, show that at the time of writing he could not have been aware of the work of the English investigators.

Prior to the publication of Hutchinson and Clayton's investigations on *Spirochaeta cytophaga*, Kellerman, McBeth, and others<sup>42</sup>, as well as McBeth and Scales<sup>31</sup>, had described a large number of aerobic bacteria which they claimed were capable of decomposing cellulose. Among these were three types, *Bac. rossicus*, *Bac. amylolyticus*, and *Bact. flavigenum*, obtained from the cultures of Omelianski's anaerobic methane- and hydrogen-producing cellulose decomposers.

For the isolation of these and other forms the American authors, in the first instance, made use of a mineral salt solution of the following composition :

Di-potassium hydrogen phosphate . . .	0.1 per cent.
Magnesium sulphate (crystalline) . . .	0.1 " "
Sodium chloride . . .	0.1 " "
Ammonium sulphate . . .	0.2 " "
Calcium carbonate . . .	2.0 " "
Tap water.	

Peptone may be substituted for ammonium sulphate.

100 c.cs. of this solution were poured into a 200-c.cs. Erlenmeyer flask in which a single sheet of filter paper, 10 cms. in diameter, was so placed as to be just covered by the solution.

The flasks thus prepared were sterilized in the usual way, inoculated with a very small quantity of the substance to be examined, and incubated at 30° C. The first signs of fermentation in this medium were the clouding of the solution followed by a dull and frayed appearance of the paper. These changes occurred after five to ten days' incubation. A small quantity of the attacked paper was at this stage removed to a control flask containing a small piece of sterile paper suspended in the mineral salt solution. If on agitation the paper from the inoculated flask broke up more readily than that of the control paper, the time had come for the inoculation of another flask, containing filter paper and mineral salt solution, with a piece of the attacked paper. After three or four transfers, carried out at the shortest possible intervals, the attacked paper was placed in a flask

with sterile water and shaken vigorously until the paper was completely broken up. From this suspension dilutions were prepared in the ordinary way for the isolation of pure cultures on one of the four types of agar described below. During incubation at 30° C. it was found most important to maintain the plates in a moist chamber to prevent the drying up of the surface of the agar. The colonies of the organism responsible for the disintegration of the paper developed slowly and the incubation had sometimes to be continued for three weeks or longer. The chief characteristic of these cellulose-decomposing forms was that they produced clear zones in the cellulose agar around the colonies. This is illustrated in Fig. 3.

The special agar media used by the American workers are prepared in the following manner:

*Cellulose agar.* To one litre of diluted ammonium hydroxide solution, containing 10 parts of ammonium hydroxide, sp. gr. 0.900, to 3 parts of water, is added a slight excess of copper carbonate. The mixture is shaken vigorously and allowed to stand overnight. After standing, the supernatant solution of cuprammonium is poured off, and in this is dissolved 15 grms. of unwashed sheet filter paper. This solution is diluted to 10 litres, and the cellulose precipitated by slowly acidifying the solution with dilute hydrochloric acid (20 per cent.). The liquid is now diluted to 20 litres, left for the cellulose to settle, and then decanted from the precipitated cellulose. This is washed with several changes of water, containing hydrochloric acid, until the washings are free from copper, and then with distilled water until free from hydrochloric acid. The cellulose precipitate is again allowed to settle for several days and is finally made up to a 1 per cent. suspension. To 500 c.cs. of this suspension are added 10 grms. of agar-agar and 500 c.cs. of the mineral salt solution already described.

*Starch agar.* 10 grms. of potato starch are suspended in 800 c.cs. of water in the cold, and the suspension boiled and stirred until it is reduced in volume to 500 c.cs. To this solution are added 10 grms. of agar-agar and 500 c.cs. of mineral salt solution as above.

*Potato agar.* To 100 grms. of mashed potatoes are added 800 c.cs. of tap water. The mixture is steamed for 30 minutes and then filtered through cotton-wool. To 500 c.cs. of this filtered liquid are added 15 grms. of agar-agar and 500 c.cs. of mineral salt solution as above.

*Dextrose agar.* 10 grms. of dextrose and 15 grms. of agar-agar

are dissolved in 500 c.cs. of tap water, and this solution mixed with 500 c.cs. of mineral salt solution as described.

The method described above for the isolation of cellulose-decomposing bacteria has also been tried by Löhnis and Lochhead<sup>54</sup>, who confirm that colonies such as described by Kellerman and his collaborators may be obtained in this way, and that the clear zones on the plates round the colonies are not due to a resolution of the calcium carbonate present in the medium, as suggested by Omelianski<sup>55</sup>. Löhnis and Lochhead further state that crude cultures of the cellulose decomposers may be obtained, in a very much shorter time than that found by Kellerman and his associates, by changing the liquid in the flask containing mineral salt solution and filter paper, as soon as it becomes turbid. Such frequent changes are claimed by them to conform more closely to the conditions existing in the animal intestine, where cellulose is constantly being decomposed by micro-organisms.

An interesting sidelight was thrown on the formation of these 'enzymatic zones', illustrated in Fig. 3, by von Gescher<sup>53</sup> in a comparatively recent paper. He confirms their formation on cellulose agar plates, but says that a microscopic examination reveals that they are not due to the enzymatic action of the colonies, but to an invasion of the medium by a large number of bacteria. Sub-cultures from the colonies had a less marked appearance, and the second or sometimes the fourth sub-culture no longer showed any zone formation. These observations show clearly that the American authors were dealing with mixed cultures on their plates and in their colonies, and that the true cellulose decomposers present in the zones could only be grown artificially through a very limited number of generations. Von Gescher's observations confirm the American investigators' statement that their cultures rapidly lost their power of decomposing cellulose.

Pringsheim and Lichtenstein<sup>56</sup>, who investigated sub-cultures of the original strains of Kellerman's organisms, were unable to confirm the views of the American authors on the physiological and cultural behaviour of these organisms.

In view of the above one hesitates to include these types



among the cellulose-decomposing bacteria, particularly since the one experiment which would have been really convincing, that of a quantitative determination of the amount of cellulose decomposed by them in pure cultures, has not yet been carried out.

A number of cellulose decomposers, which appeared to be readily isolated, were described by Sack<sup>57</sup> in 1924. He obtained them from filter paper buried a few centimetres deep in the soil or in mud.

When the paper had been exposed for a few weeks it was washed with sterile water and placed in a flask containing a solution of di-potassium hydrogen phosphate and potassium nitrate, with a piece of filter paper. When this paper had become attacked after incubation at a suitable temperature, a loop full of liquid was smeared over three ordinary agar plates. The colonies appearing on these plates were used to inoculate fresh flasks containing filter paper and the above inorganic nutrient solution. On the appearance of signs of destruction of the filter paper in these flasks, agar plates were again smeared from the liquid as before, and the developing colonies used to inoculate further flasks. This procedure was continued until the colonies appearing on subsequent plates were identical with those used as inoculant.

In this way Sack states that he succeeded in obtaining pure cultures of his rods, two from soil and one from mud, and a coccus, also from soil, and he remarks that the ability of these types to destroy cellulose has been retained for more than a year in their sub-cultures. The three rods he places in the genus *Cellulomonas* of Bergey<sup>58</sup>, and the coccus in a new genus *Cellulococcus*.

That the technique adopted by Sack is by no means faultless, any one acquainted with bacteriological methods will observe from a perusal of his paper. At no stage does he attain a progressive elimination of any infection forms which undoubtedly must have been present in the first instance. Until his method has been subjected to a more stringent technique it is not considered justifiable to accept his four types as true cellulose decomposers.

In a second paper<sup>59</sup> the same author records the isolation in pure culture of four nitrate-producing bacteria, which he claims to be capable of decomposing cellulose. Here, again,

confirmation of his work appears to be required before the claim can be accepted.

Recently Gray and Chalmers<sup>2</sup> have described a further type of aerobic micro-organism capable of decomposing cellulose and gelose, under the name *Microspira agar-liquefaciens*.

This is a short, curved rod, usually C-shaped, measuring 0.5 to 0.7  $\mu$  by 2  $\mu$ . Sometimes the organism resembles a coccus in appearance. It is vigorously motile in young cultures by means of a terminal flagellum, and is strictly aerobic. Its optimum temperature for growth is 25°C., and it does not develop at temperatures higher than 30 to 32°C.

It grows well on the ordinary agar media, and liquefies agar, if more readily decomposable carbohydrates, such as glucose, are absent. Cellulose suspended in a mineral solution of 0.1 per cent. potassium nitrate, 0.1 per cent. di-potassium hydrogen phosphate, 0.02 per cent. crystalline magnesium sulphate, 0.01 per cent. sodium chloride, 0.01 per cent. calcium chloride, and 0.002 per cent. ferric chloride, is rapidly decomposed at the surface of the liquid. On Kellerman's cellulose agar no growth takes place.

It is of special interest that small amounts of xylose or lignin, added to the medium in which *Microspira agar-liquefaciens* is grown, increase the amount of cellulose decomposed by the organism.

An interesting group of aerobic bacteria was described by Groenewege<sup>60</sup> in 1920 under the name of *Bact. cellaresolvens*  $\alpha$ ,  $\beta$ , and  $\gamma$ . These organisms, which do not decompose cellulose when grown in pure culture, are claimed to do so when living symbiotically with denitrifying forms belonging to the *Bact. fluorescens* group. Two of these were described by Groenewege under the names of *Bact. opalescens* and *Bact. viscosum*. *Bact. cellaresolvens* may be isolated from filter paper which has been buried in soil about 15 cms. deep and left there to rot.

The partly decomposed paper is washed frequently with sterile water to remove the bulk of the other micro-organisms adhering to it, and some of the washed fibres are then wiped over the surface of an ordinary agar plate with the aid of a sterile triangular glass rod known as a Drigalski rod.

After five to six days' incubation of the plates, small colonies of *Bact. cellaresolvens* appear. These decompose

the paper when grown symbiotically with one of the two denitrifying forms in a medium composed of di-potassium hydrogen phosphate 0.25 per cent., potassium nitrate 0.6 per cent., filter paper 2 per cent., and tap water.

The existence of such forms as *Bact. cellaresolvens* shows that the natural disposal of waste cellulose under certain conditions may cause the destruction of one of the most important foods, the nitrates, and thus be harmful rather than beneficial to the soil. In view of the established fact (Koch and Pettit<sup>61</sup>) that denitrification is of very little importance in well aerated soils containing less than 25 per cent. of moisture, it is not likely that the denitrification caused by the symbiotic group described by Groenewege can be of serious importance in nature.

Now and again statements appear in the literature (e.g. Trotman and Sutton<sup>62</sup>) that forms such as *Bac. mesentericus* and *Bac. subtilis* are capable of decomposing cellulose. Sufficient proof for these statements has, however, not yet been brought forward.

More interesting is the account by Merker<sup>63</sup> of two aerobic species of cocci which were found on living leaves of *Elolea*, where they produce a gradual destruction of the cell walls. Neither of them was obtained in pure culture, since they were found to be incapable of development on ordinary laboratory media. The cultures must have been almost pure, however, judging from the uniformity of their physiological reactions.

The more active type is described by Merker under the name of *Micrococcus cytophagus*. When inoculated into a medium containing filter paper it covers the cellulose with a transparent vitreous and yellow growth, which on microscopical examination is found to consist of a zoogloea of somewhat egg-shaped cocci. Lignified or suberized tissues are not attacked by it. As the destruction of the cellulose proceeds, the paper collapses to form an amorphous mass at the bottom of the container.

*Micrococcus melanocyclus* was obtained from a crude culture of *Micrococcus cytophagus*. It destroys cellulose less extensively than the latter, and when inoculated into a medium

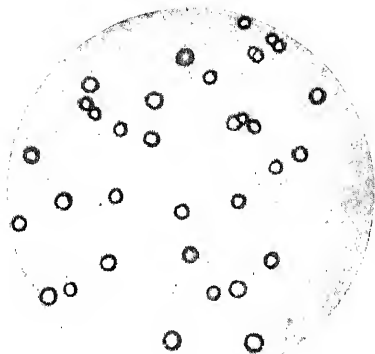


FIG. 3. Colonies of an aerobic cellulose-decomposing bacterium of the types described by Kellerman and his collaborators, after 15 days' growth at 30° C. on cellulose agar. Natural size. (Taken from Bulletin No. 266 of the U.S. Department of Agriculture, Bureau of Plant Industry.)



FIG. 4. An advanced stage in the resolution of a cellulose fibre by *Bac. methanigenes*, showing spore formation in various stages of progress. Magnification  $\times 1,000$ .



containing strips of filter paper covers the latter with black concentric rings. In young cultures a red coloration of the attacked paper sometimes occurs, the colour gradually changing to black. The pigment of both of these cocci is regarded by Merker as related to carotin.

It is highly probable that on further investigation facultative and obligatory anaerobic cocci may be found to take a very active part in the decomposition of cellulose. Henneberg's<sup>64</sup> investigation on the destruction of plant tissue in the intestine of man supports this view.

(b) The facultative anaerobic forms.

1. **Denitrifying forms.** Investigations carried out by van Iterson, jr.<sup>51</sup>, on the microbiological decomposition of cellulose revealed that mineral salt solutions, containing di-potassium hydrogen phosphate and potassium nitrate in addition to filter paper, gave rise to an evolution of free nitrogen and caused a breakdown of the cellulose when inoculated with ditch mud and incubated at about 30° C. This fermentation, however, was not studied in detail as regards the responsible micro-organisms, and it was not established whether the cellulose-destroying forms were also responsible for the denitrification, or whether symbiotic reactions were taking place in the fermenting liquid similar to those observed by Pringsheim<sup>65</sup> in the case of the bacteria responsible for the fixation of nitrogen and those causing the destruction of cellulose. It is still to be established, therefore, whether bacteria exist which liberate nitrogen from nitrates or nitrites during the decomposition of cellulose. It is also an open question whether such forms, if they do exist, should be classed among the facultative or the obligatory anaerobes.

(c) The obligatory anaerobic forms.

1. **Denitrifying types.** Information is lacking as to the existence of such forms beyond van Iterson's observations already quoted.

2. **Types which do not liberate atmospheric nitrogen.** To

this group belong first and foremost the two classical cellulose decomposers *Bac. methanigenes*, Lehmann and Neumann, and *Bac. fossilicularum*, Lehmann and Neumann, isolated by Omelianski<sup>8</sup> towards the close of the last century. These organisms were the first bacteria definitely shown to be capable of decomposing cellulose in the form either of filter paper or of cotton-wool.

Both organisms are found in places where plant tissues decay under anaerobic conditions, as for instance in dung or in the mud of ponds and rivers.

From these sources they may be obtained in artificial culture. For their isolation Omelianski used horse dung, or mud from the river Neva, inoculated into flasks completely filled with a medium containing, in addition to filter paper, the following mineral salt solution: di-potassium hydrogen phosphate 0.1 per cent., magnesium sulphate (crystalline) 0.05 per cent., ammonium sulphate or phosphate 0.1 per cent., sodium chloride a trace, calcium carbonate 1 per cent., and distilled water. The ammonium phosphate or sulphate could be replaced by 0.1 per cent. of peptone or asparagin.

The inoculated flasks are incubated at 34 to 35° C., the optimum temperature. A fermentation started in this way does not begin to give off gas until about eight days after incubation. At first the gas consists of a mixture of hydrogen, methane, and carbon dioxide, but after several sub-cultures into fresh media of the above composition the amount of hydrogen given off diminishes, and finally the gas consists of a mixture of methane and carbon dioxide only. The ratio of methane to carbon dioxide varies somewhat with the age of the culture, and may reach 75 parts of methane to 25 parts of carbon dioxide while the culture is young. This is probably due in part to the greater solubility of carbon dioxide in water and to the formation of calcium bicarbonate. At this stage, when the gas evolved is free from hydrogen, the culture is comparatively pure, but it may be further purified by pasteurization before inoculation into fresh media, provided that the spores of the methane bacillus are present at the time of pasteurization. In this way all the non-spore-forming types of infections are removed.

A culture prepared in this way and examined under the microscope, shows a uniform growth of a long and very slender rod, measuring about  $0.4\ \mu$  in width by  $5\ \mu$  in length, and taking the usual stains fairly readily. The rods are found deposited in large numbers on the surface of the cellulose fibres. In consequence, many of them are curved or bent, since they retain the outline of the section of the fibre on which they were deposited, even after being washed off the fibre. As the culture becomes older, and the decomposition of the cellulose progresses, the slender rods grow longer. Finally, they reach up to three times their original length. A more deeply staining swelling now appears at one end of the rod. In this swelling a circular spore is formed which, when ripe, measures  $1\ \mu$  in diameter. It is at this stage of spore formation that *Bac. methanigenes* shows the most characteristic appearance. Fig. 4 shows a photomicrograph of the organism at this phase.

As will be seen from this photograph the resolution of the fibres has already reached an advanced stage. The progress of the destruction may also be followed macroscopically. As the attack proceeds the filter paper, if this source of cellulose is used, becomes orange, or yellowish-orange, and shows numerous holes, and frequently also a much-frayed edge. Finally, it loses its fibrous structure and collects at the bottom of the flask as a blackish or yellowish-orange sediment. The decay progresses slowly and may continue for several months without interruption. While the fermentation continues briskly the output of mixed gases varies between 3.5 c.cs. and 15.5 c.cs. per gramme of cellulose. In addition to the gases, volatile fatty acids are produced during the fermentation. Omelianski<sup>66</sup> gives the following figures for a cellulose fermentation by *Bac. methanigenes*:

<i>Cellulose.</i>		<i>Fermentation Products Recovered.</i>	
	grms.		grms.
Used for the experiment	2.0815	Fatty acids . . .	1.0223
Remaining as residue	0.0750	Carbon dioxide . . .	0.8678
Decomposed during		Methane . . .	0.1372
fermentation . . .	<u>2.0065</u>	Total	<u>2.0273</u>

The slight excess of recovered fermentation products, over and above the weight of cellulose decomposed, is regarded by Omelianski as within the limits of experimental error.



The fatty acids formed consist of a mixture of acetic and butyric acids, in the proportion of nine molecules of acetic to one molecule of *n*-butyric acid. Aldehydes, ketones, or alcohols are not formed during the fermentation.

The quantity of methane evolved per gramme of cellulose decomposed was found by Omelianski to be at least equal to that collected by Schlösing<sup>67</sup> from a fermenting manure heap, and quite sufficient to explain the accumulation of marsh gas in the mud of stagnant ponds, where it may be seen rising to the surface in large bubbles.

Neither from *Bac. methanigenes* nor from *Bac. fossicularum*, which will be described below, did Omelianski succeed in obtaining pure cultures in the usual way, since both organisms failed to develop on the ordinary laboratory media, such as agar or gelatine. That the culture with which Omelianski worked must have been sufficiently pure to justify the assumption that the organism was chiefly responsible, not only for the actual breakdown, but also for the formation of the products found, there can be no doubt, since the microscopic preparation of the culture, after the completion of the fermentation, showed a uniform field without the presence of infection forms. Kellerman and McBeth's<sup>42</sup> statement that *Bac. methanigenes* and *Bac. fossicularum* are not responsible for the production of the methane and hydrogen respectively is not justified, therefore, and the fact that these investigators succeeded in isolating three other types of micro-organisms from Omelianski's cultures does not invalidate this conclusion. Since Omelianski's own investigations, many attempts have been made to obtain pure cultures of these organisms, but so far without success. It is possible that better results might be obtained by utilizing gum arabic instead of cellulose for this purpose. Omelianski himself states that gum arabic is a suitable carbohydrate for his organisms, and the writers of this volume have now and again succeeded in obtaining woolly, and very coherent, colonies of what would appear to be *Bac. methanigenes*, by using gum arabic instead of cellulose for the isolation. Further investigations, however, are required to establish the nature of these colonies.

According to Tappeiner's<sup>68</sup> investigations the methane-producing cellulose fermenters develop best under acid conditions, the hydrogen-producing types preferring a slightly alkaline reaction.

*Bac. fossicularum*, Omelianski's hydrogen-producing cellulose decomposer, is associated with *Bac. methanigenes* wherever the latter occurs, and can therefore be obtained from mud. The rate of germination of its spores is slower than that of the spores of the methane bacillus, and this fact was utilized by Omelianski for the separation of the two. It was mentioned above that the gas evolved by the first crude culture prepared for the isolation of *Bac. methanigenes* contained hydrogen as well as methane. If such a culture is heated to 75° C. for fifteen minutes shortly after the gas evolution has commenced, the vegetative cells of the methane bacillus which have by then developed from their spores are destroyed, whereas the spores of the hydrogen bacillus, not germinated, remain unaffected by the heat. Repeating this treatment through three or four successive generations from the original crude culture, a culture is obtained which gives off a gas containing hydrogen and carbon dioxide only. It is interesting to note that when the methane organism is subjected to the same treatment it is eventually destroyed. This disposes of the possibility that the organism responsible for the evolution of the mixture of methane and hydrogen can be one and the same type, which through subjection to a high temperature loses its property of producing methane, but maintains its faculty of evolving hydrogen. That the two organisms are not the same species is also indicated by their difference in size.

*Bac. fossicularum* measures 0.5  $\mu$  in width and in young cultures from 4 to 8  $\mu$  in length. In older cultures it reaches a length of from 10 to 15  $\mu$ . Its spores have a diameter of 1.5  $\mu$  against 1  $\mu$  in the case of *Bac. methanigenes*. Like the latter, the vegetative cells are non-motile and do not stain blue or purple with iodine, the characteristic staining reaction for *Bac. amylobacter*.

The hydrogen fermentation of cellulose progresses more slowly than the methane fermentation, and at its optimum temperature (35° C.) may continue uninterruptedly for more

than a year. The gas, consisting of hydrogen and carbon dioxide, is produced at a slower rate and amounts on an average to 8 c.c.s. per gramme of cellulose during 24 hours. As in the case of the methane fermentation, the ratio of the gases varies according to the age of the fermentation. It may amount to 80 per cent. of hydrogen and 20 per cent. of carbon dioxide at the beginning of the fermentation and 4.5 per cent. and 95.5 per cent. respectively immediately afterwards. Later, the hydrogen content of the gas gradually rises to 32 per cent. and then falls to 20 per cent. and less towards the end of the fermentation.

The following balance sheet is given by Omelianski<sup>66</sup> for the hydrogen fermentation:

<i>Cellulose.</i>		<i>Fermentation Products Recovered.</i>	
	grms.		grms.
Used for the experiment	3.4743	Fatty acids . . .	2.2402
Remaining as residue .	0.1272	Carbon dioxide . . .	0.9722
		Hydrogen . . .	0.0138
		<i>Total</i>	3.2262
Decomposed during fermentation . .	3.3471	Unidentified compounds	0.1209
			3.3471

In addition to the fatty acids, consisting of 1.7 mol. of acetic to 1 mol. of butyric acid, traces of valeric acid and of higher alcohols are formed, which, with the colouring matter and the aromatic substances giving the culture a smell of cheese, explain the remaining 0.1209 gramme of cellulose not accounted for in the above calculation.

It remains to be mentioned that macroscopically *Bac. fossilicularum* causes exactly the same changes in the appearance of the cellulose fibres as those described in the account of the methane fermentation.

Omelianski's researches on the methane fermentation of cellulose have been confirmed by many investigators, and as recently as 1916 Oechsner de Coninck<sup>69</sup> found a small amount of *n*-propionic acid among the fermentation products of these bacteria in addition to acetic and *n*-butyric acids in the proportions stated by Omelianski.

In 1923 Khouvine<sup>70</sup> published a description of yet another cellulose decomposer, *Bac. cellulosa dissolvens*, which is of special interest, since in addition to other products it yields about 8 per cent. of ethyl alcohol, calculated on the cellulose fermented. Morphologically, *Bac. cellulosa dissolvens* greatly resembles Omelianski's *Bac. fossicularum*, but its spores are oval instead of circular and measure  $2\mu$  by  $2.5\mu$ . The organism develops well at temperatures up to  $57^{\circ}\text{C}$ ., and in this respect it represents a transition stage to the thermophilic cellulose decomposers. For its isolation, Khouvine tried without success a variety of media recommended for the cultivation of cellulose decomposers, including those used by Kellerman and his collaborators.

Finally, a mineral salt solution was adopted which consisted of 0.1 per cent. di-potassium hydrogen phosphate, 0.1 per cent. sodium chloride, 0.1 per cent. pancreatic peptone, filter paper, and tap-water, to which was added a 10 per cent. aqueous extract of faecal matter, sterilized at  $110^{\circ}\text{C}$ . for 15 minutes, in the proportion of 250 c.cs. of extract to 750 c.cs. of mineral salt solution.

Grown anaerobically in this medium, the organism could be gradually freed from its various infection forms, but pure cultures from isolated colonies were not obtained. *Bac. cellulosa dissolvens* does not develop on any of the ordinary laboratory media, and does not ferment any carbohydrates but cellulose.

The following balance sheet for a typical fermentation by this organism is given by Khouvine:

	grms.	Products Recovered.	grms.
Cellulose fermented	1.012	Acetic acid . . .	0.275
		(?) <i>n</i> -Butyric acid . . .	0.033
		Ethyl alcohol . . .	0.082
		Carbon dioxide . . .	0.1827
		Hydrogen . . .	0.0085
		Pigment . . .	0.0135
		Total	0.5947
		Unrecovered products .	0.4173
			<u>1.0120</u>

Only 55.15 per cent. of the carbon fermented was recovered in the isolated fermentation products. Khouvine regards the remainder as being present in the fermentation liquid in the form of soluble carbohydrates, which are capable of becoming absorbed by the intestine.

### B. The thermophilic bacteria.

With this group a field is again entered which is comparatively unexplored. In 1899 MacFayden and Blaxall<sup>71</sup> recorded that the thermophilic bacteria which they had observed to be present in soil were able to ferment cellulose in the form of Swedish filter paper and viscose, the disintegration of these types of cellulose requiring about 21 days to become complete at 60° C. The decomposition products of this fermentation comprised acetic and butyric acids, as well as carbon dioxide and methane. The organisms used were not isolated in pure culture. Similar organisms were investigated in greater detail by Kroulik<sup>72</sup> in 1912. Though he was unable to obtain pure cultures, his microscopic investigation of the types convinced him that the decomposition of the cellulose was caused by two distinct forms, described under the names *Bacillus II, 1* and *Bacillus II, 2*.

Of these, *Bacillus II, 1* is an aerobic (facultative anaerobic) form. Its large oval spore germinates to form a fairly broad cell which often attains a considerable length. On ageing, this cell divides into a number of shorter or longer individuals. At this stage spore formation sets in, each cell showing one or two terminal spores. On ripening of the spores the vegetative cell is resolved.

*Bacillus II, 2* is stated to be facultatively anaerobic. It forms a small spherical spore which on germination gives rise to a long very slender thread, which after subdivision again produces terminal spherical spores. This latter type, therefore, resembles *Bac. methanigenes* in its morphology.

Both of these thermophilic bacteria decompose cellulose with the production of formic, acetic, and butyric acids, and with an evolution of carbon dioxide and hydrogen, the latter in comparatively small amounts. The presence of methane was not observed. The sulphuretted hydrogen which was

occasionally formed in appreciable quantities was regarded as a product of interaction between the hydrogen evolved and the sulphates present in the medium. The destruction of the cellulose was more extensive when *Bac. II*, 2 was used, reaching 90 per cent. or more of the total cellulose.

In 1923 Langwell and Hind<sup>73</sup> gave an account of a thermophilic cellulose-decomposing bacterium which was stated to produce volatile organic acids, ethyl alcohol, methane, hydrogen, and carbon dioxide. A description of this organism, together with a balance sheet for the reactions, was given. It is understood, however, that these statements have not been confirmed on further investigation, and that pure cultures of the organism have not yet been obtained.

In 1924 Fred, Peterson, and Viljoen<sup>74</sup> studied the breakdown of cellulose by another thermophilic type of which a morphological description has quite recently appeared<sup>75</sup>. Though the authors mention in this second paper that they have succeeded in isolating the type in pure culture, it is very questionable whether independent investigation would confirm this, since Viljoen, Fred, and Peterson obtained the culture they describe as pure from a dilution containing as much as one ten-thousandth part of the crude culture, and from this dilution in cellulose agar they isolated not one well-circumscribed colony, but were content to employ as inoculant for their 'pure cultures' a part of this agar medium containing a gas bubble. Even starting with a culture that is only slightly infected it is highly improbable that the above procedure could lead to the isolation of pure cultures.

The data which Viljoen, Fred, and Peterson supply as to the properties of their cellulose fermenter, for which they suggest the name *Clostridium thermocellum*, cannot therefore be accepted as final. The interest which for many reasons attaches to the study of the thermophilic cellulose fermenters makes it highly desirable that these data should be verified on undeniably pure cultures. In the earlier paper referred to above, Fred, Peterson, and Viljoen reported that when grown at a temperature between 62 and 66° C. their organism was found to decompose cellulose rapidly, yielding as much as

56.8 per cent. of acetic acid and 10.3 per cent. of ethyl alcohol from the cellulose decomposed, after eleven days' incubation. By this time 60 to 80 per cent. of the cellulose disappeared, while the remainder formed a yellowish structureless sediment. In the second publication the yield of alcohol is stated to vary very considerably.

Before leaving this interesting group of thermophilic cellulose decomposers, it should be mentioned that many of the earliest investigators of the natural decomposition of vegetable matter, besides MacFayden and Blaxall, no doubt dealt with the breakdown caused by thermophilic bacteria. Interest at the time, however, was centred more on the surprising observation that micro-organisms may live at temperatures above the coagulation point of protein than on the biochemical reactions of the types, and consequently this aspect of the physiology of the thermophilic micro-organisms was neglected.

## ORDER II. The myxobacteriales.

The myxobacteriales, a small group of schizomycetes, were first described by Thaxter<sup>76</sup>. Like the myxomycetes, their life cycle is divided into a stage of aggregation, in which the cells accumulate to form a slimy mass without, however, losing their individuality, and a cyst, or resting stage, in which several of the organisms are encased in a cyst or spore-like body. The individual cell of the myxobacteria is a rod, which usually measures about  $15\mu$  in length. It multiplies by fission and is slowly motile, without, however, possessing any flagella.

The myxobacteria are generally found living on decaying wood and on dung. Whether they take an active part in the breakdown of the vegetable tissues, through the decomposition of cellulose, hemicelluloses, pectin, or gums, has not yet been established.

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## CHAPTER IV

### THE ACTINOMYCETES

THE fragmentary and often diverse descriptions of the actinomycetes, or ray fungi, which are found in the usual text-books on microbiology, make it desirable to discuss the morphology of these organisms in some detail before proceeding to deal with those types among them which have been found capable of decomposing cellulose and its associated substances. This description of the morphology of the ray fungi is based on Ørskov's<sup>1</sup> recent investigations of the group.

An explanation is also necessary of the adoption of the name actinomycetes to describe the whole of this group in preference to the other names, such as *Streptothriceae*, *Nocardiaceae*, or Discomycetes, which have been suggested from time to time. In this respect the lucid exposition by Breed and Conn<sup>2</sup> on the history of the nomenclature of the ray fungi has been followed. According to these authorities the first member of the group to be studied was named *Streptothrix Foersteri* by Cohn in 1875. The name of *Streptothrix*, however, had already been used by Corda in 1839 to describe quite a different fungus, which to-day comprises several species. The application of the name *Streptothrix* to the ray fungi was, therefore, systematically misleading. In 1878 Rivolta suggested the name of *Discomyces* for a ray fungus, a name which he later repudiated in favour of that of *Actinomyces lovis*, given to the same organism by Harz in 1877. This latter name has sometimes been confused with that of *Actinomyce* used by Meyen in 1828 for an eumycete. This eumycete, Breed and Conn state, was later recognized by Meyer as identical with *Tremella meteorica*, Persoon. The name of *Actinomyces* is, therefore,

free and can be used with justification both for the type described by Harz and as a group name for all those ray fungi for which *Actinomyces bovis*, Harz, serves as the type.

To avoid misunderstanding in the nomenclature of the ray fungi, Trevisan suggested in 1889 another name, *Nocardia*, for these organisms. This name is sometimes met with in the literature, but there is little justification for it, since the name of *Actinomyces* is not only legitimately used, but is older. How far it is justifiable to retain the name of *Actinomyces* for all the ray fungi, as has been done in these pages, may be debatable, since they include forms for which *Actinomyces bovis*, Harz, cannot serve as the type. However, since a close relationship probably exists between these forms and *Actinomyces bovis*, it has been thought preferable to adhere to the well-known name of *Actinomyces* until such time as the whole group shall have been thoroughly investigated, and all the facts have been made clear, rather than to add to the existing confusion by introducing yet another name.

Basing his system on morphological characters, Ørskov divides the ray fungi into three groups, of which the second is again divided into two sub-groups A and B.

**Ørskov's Group I.** In his first group, to which *Actinomyces bovis*, Harz, belongs, the organisms form a unicellular, slender, and profusely branched mycelium, radiating evenly from the centre, and sending branches into the medium on which they grow, thereby causing the colonies to adhere firmly to the substratum. The hypha of the mycelium measures from  $0.5\ \mu$  to  $1.0\ \mu$  in diameter. The whole structure of the mycelium gives to the colonies a tough leathery appearance. From the ground mycelium rise, sooner or later, somewhat thicker hyphae, the aerial hyphae, measuring about  $2\ \mu$  in diameter. These are sometimes twisted into spirals, but in other cases are straight. Sometimes they are branched like the ground mycelium. From the aerial hyphae the spores are produced. When spore formation takes place, the plasma of the aerial hypha contracts into even segments, separated by what appear to be empty spaces. The cell wall of the aerial hypha gradually sinks into these spaces, thereby giving the

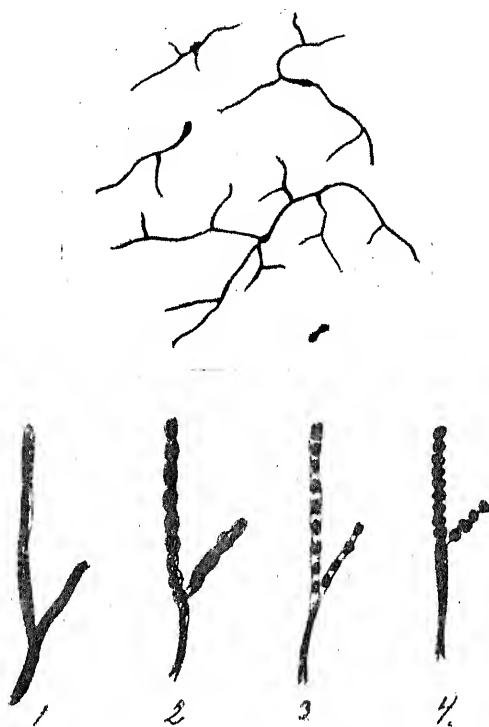


FIG. 5. Aerial hyphae in process of spore formation and germinated spores of an actinomycete, belonging to Group I, Ørskov. (From J. Ørskov, *Investigations into the Morphology of the Ray Fungi*.)



hypha a beaded appearance. When the spores are ripe their plasma is entirely surrounded by the cell wall of the hypha. At no stage in the formation of the spore is it possible to discover a schizogen-formed wall, such as is found in the schizomycetes.

The aerial hyphae with their thin ripe spores cover the whole or a part of the colonies, often in annular layers, and appear macroscopically as a fine dust, which is whitish or grey in many cases. The spores show a somewhat greater resistance to high temperatures than the mycelium, though the difference is not nearly as marked as in the case of the spores of bacteria. The formation of aerial hyphae and spores is sometimes delayed, as for example, when the organism develops on a medium rich in food substances. This fact is probably responsible for the statement sometimes made that forms belonging to this group produce no aerial hyphae. In such cases Ørskov recommends the cultivation of the organism on a medium deficient in food, for example on a water agar composed of tap-water in which 2 per cent. agar-agar has been dissolved. On a medium such as this, spore formation invariably takes place within a few days.

It is also characteristic of this first group that the spore, on germination, shows little tendency to swell or grow, and is, therefore, far less prominent in a young mycelium than in the case of the so-called 'spores' of the members of the second group, which is discussed below.

Grown in liquid culture the actinomycetes of Group I develop at the bottom of the container. If the layer of liquid is comparatively shallow the mycelium may finally spread throughout it and reach the surface, where aerial hyphae and spores are then formed.

To Group I belong many of the saprophytic actinomycetes so frequently met with in the soil.

In Fig. 5 are shown aerial hyphae in process of spore formation and germinated spores with young mycelium of an actinomycete belonging to this group.

**Ørskov's Group II.** The actinomycetes of Group II are less constant in their characters than those of Group I. The

group is divided into two subdivisions, depending on the presence or absence of an aerial mycelium.

When the spore of an actinomycete belonging to *sub-group A* germinates it continues to grow for some time and in consequence appears larger than the hyphae of the young mycelium. The mycelium is polymorphous, being more irregular in size and shape than is the case in Group I. Aerial hyphae, which are indistinguishable from the hyphae of the mycelium, are always formed at an early stage of the development. As the organism grows older both the aerial hyphae and the ground mycelium become divided into uneven segments. The lateral walls responsible for the division start as circular rings on the inside of the wall of the hypha and gradually extend towards the centre until complete transverse walls are formed. This method of cell division is quite different from that observed among the schizomycetes. The segments of the aerial hyphae formed in this manner can hardly be regarded as real spores, since they show no greater resistance to heat than do the segments of the ground mycelium or the young undivided mycelium itself. Grown in liquid culture the actinomycetes of *sub-group A* are stated to develop both at the bottom of the container and on the surface of the liquid.

The most remarkable feature of the actinomycetes of *sub-group B* is their so-called 'angular growth', that is, their formation of V- and Y-shaped cells. Such cells are formed by the incomplete separation of the fragments of hyphae after segmentation. The transverse walls of adjacent segments remain hinged at one side and develop a tendency to swell, thereby forcing the segments apart, except at the joined places. The V- and Y-shaped cells give the growth of these actinomycetes the appearance of preparations of cultures of *Corynebacterium diphtheriae*. Fig. 6 shows a typical example of such angular growth.

The actinomycetes of *sub-group B* very often form little or no mycelium, and branching of the mycelium is rare. No aerial hyphae are formed. When grown in liquid culture, development takes place both at the bottom and on the surface of the liquid. As the organisms of this group usually



FIG. 6. Angular growth of an actinomycete belonging to Group II B, Ørskov.  
(From J. Ørskov, *Investigations into the Morphology of the Ray Fungi.*)

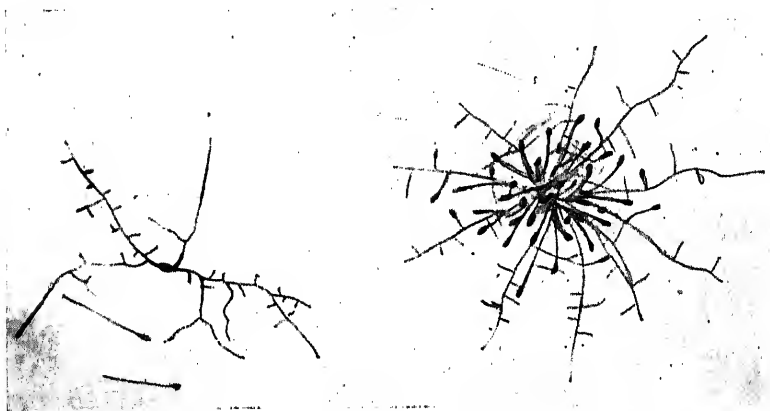


FIG. 7. *Actinomyces chaliceae*, germinating spore and young colony.





lack a true mycelium their colonies are generally much softer than those of the other groups and can be removed from the substratum without difficulty.

**Ørskov's Group III.** The third main group of actinomycetes is represented in Ørskov's account by only one type, *Actinomyces (Streptothrix) chalceae*. This organism forms a delicately branched and unseptate mycelium. At the end of the branches a single spore is formed. Fig. 7 illustrates this organism.

Grown in liquid cultures, *Actinomyces chalceae* develops at the bottom of the container only.

Basing their classification on physiological rather than on morphological properties, Waksman and Curtis<sup>3</sup> have attempted to construct a system for the grouping of a number of actinomycetes investigated by them. Their system, however, overlooks the important morphological differences described by Ørskov and can only be regarded as a temporary expedient. The same is undoubtedly the case with the somewhat more elaborate system of classification proposed by the Society of American Bacteriologists and given in Bergey's *Manual of Determinative Bacteriology*<sup>4</sup>. Though this latter system pays some attention to differences in morphological structure, it is still much inferior to Ørskov's grouping in this respect. Thus, in the American system, forms belonging to Ørskov's Group II, B, that is, those producing no aerial mycelium and showing typical angular growth, are grouped with types showing the characteristic development of the unicellular actinomycetes of Group I of Ørskov.

Until a really satisfactory system has been evolved it would appear desirable, in describing new species, first of all to pay attention to morphological characters, for instance on the basis of the system recommended by Ørskov, and then, where necessary, to utilize the biochemical reactions of the species for their further subdivision.

After this digression, it is possible to proceed to a consideration of the extent to which the actinomycetes are capable of decomposing cellulose, hemicelluloses, pectin, and gums. Positive evidence in this respect has been forthcoming only

within the last decade, though earlier investigations had made it probable that these substances constituted suitable sources of carbohydrates for the group. Beijerinck<sup>5</sup> was one of the first to associate the actinomycetes with the decay of vegetable matter in his study of the physiology of *Actinomyces chromogenes*, Gasparini. He found this organism widely distributed in soils, particularly on and in dead cells of the primary cortex of the roots of trees such as oak and beech, and on the rhizomes of ferns. Because of its quinone production he regarded *Actinomyces chromogenes* as an active agent in humus formation. Hiltner and Störmer<sup>6</sup> observed an increase in the number of actinomycetes in a fallow soil after dressing with farmyard manure, and suggested that they were active in the decomposition of the straw of the manure. In a later investigation Störmer<sup>7</sup> reported that *Actinomyces chromogenes* develops well on humic substances.

In 1914 Krainsky<sup>8</sup> reported on an investigation on the action of a number of actinomycetes on resistant cellulose. From the data given the following species appear to be capable of decomposing this carbohydrate, when grown in the medium detailed below:

	grms.
Ammonium chloride . . . . .	0.05
Di-potassium hydrogen phosphate . . . . .	0.05
Cellulose . . . . .	2.0
	c. cs.
Tap-water . . . . .	100

*Actinomyces cellulosa*, Krainsky, belonging to Group I Ørskov.

Has spherical spores measuring about  $1.3\ \mu$  in diameter. The colonies on agar are yellowish, with a whitish to grey aerial mycelium. On potato the growth is greyish, with grey aerial mycelium. A soluble yellow pigment is produced by the organism. Gelatine is liquefied and starch hydrolysed.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces diastaticus*, Krainsky, belonging to Group I Ørskov.

Produces oval spores,  $1.0$  to  $1.2\ \mu$  by  $1$  to  $1.5\ \mu$ . The aerial hyphae bearing them may form long delicate spirals. The colonies on agar are cream coloured, thin, and spreading; on potato, growth abundant, wrinkled, cream coloured, but with a greyish tinge. The aerial mycelium is usually white, later becoming

drab. A brown to dark brown soluble pigment is formed. Gelatine is liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces flavochromogenus*, Krainsky, syn. *Actinomyces chromogenes*, Gasparini, belongs to Group I Ørskov.

Its colonies on agar are yellowish-grey with a white to grey aerial mycelium. On potato, growth is similar to that on agar, and a white aerial mycelium is formed. The species produces a dark brown soluble pigment. Gelatine is slightly liquefied and starch slightly hydrolysed.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces flavus*, Krainsky, probably belonging to Group II Ørskov.

The growth consists of coarse branching hyphae which break up to form oval spores on ageing. The colonies on agar are grey, spreading and somewhat wrinkled. Growth on potato is raised, much wrinkled, and greenish-olive in colour. A brown soluble pigment is formed. Gelatine is liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces griseoflavus*, Krainsky, belongs to Group I Ørskov.

The growth on agar is yellowish with white aerial mycelium; on potato the growth is yellowish with grey aerial mycelium. The spores are spherical to oval,  $1.2\ \mu$  in diameter. Gelatine is rapidly liquefied, starch slightly hydrolysed.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces griseus*, Krainsky, belongs to Group I Ørskov.

On agar an abundant cream coloured and transparent growth is formed. On potato the growth is yellowish and wrinkled. The aerial mycelium is water-green. The rod-shaped to short cylindrical spores measure  $0.8\ \mu$  by  $0.8$  to  $1.7\ \mu$  and are formed on hyphae which occasionally form spirals. A soluble pigment is not formed. Gelatine is rapidly liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces melanocyclus*, (Maerker) Krainsky, belongs to Group I Ørskov.

Growth on most media is poor; the colonies formed are orange-red with a black aerial mycelium, formed near the edge of the colonies. The spores are almost spherical and measure  $0.9\ \mu$  in diameter. Gelatine is liquefied and starch hydrolysed.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces melanosporus*, Krainsky, belonging to Group I Ørskov.

The growth on most media is reddish with black aerial mycelium.

The spherical spores measure  $1.2\ \mu$  in diameter. Gelatine is liquefied, starch slightly hydrolysed.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces microflavus*, Krainsky, belongs to Group I Ørskov.

It forms a yellow growth with rose-yellow aerial mycelium on agar. On potato, growth is yellow, but no aerial mycelium is formed. The spores are spherical to rod-shaped and measure  $2\ \mu$  by 2 to  $5\ \mu$ . Gelatine is liquefied and starch hydrolysed.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces parvus*, Krainsky, belongs to Group I Ørskov.

The growth is yellowish on most media with a light yellow aerial mycelium. The spores are more or less spherical, measuring  $1.6\ \mu$  in diameter. Gelatine is slowly liquefied, starch hydrolysed.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces roseus*, Krainsky, belongs to Group I Ørskov.

On agar the colonies are white, becoming yellowish on ageing. On potato the growth is brownish and much wrinkled. The aerial mycelium is pale brownish. The hyphae on which spores are formed show numerous open and closed spirals. The spores are oval, measuring  $1.0$  to  $1.2\ \mu$  by  $1.5$  to  $3\ \mu$ . Gelatine is liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces ruber*, Krainsky, belongs to Group I Ørskov.

The colonies on agar are raised, wrinkled, and olive-green; on potato, growth is similar. A brown soluble pigment is produced. The aerial mycelium is chrome-orange and woolly. The measurements of the spores are not given. Gelatine is liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

An investigation of the action of actinomycetes on cellulose was reported on in 1919 by Waksman and Curtis<sup>9</sup>. Those of the species studied by them which are recorded by Bergey<sup>4</sup> are described below:

*Actinomyces albus*, (Krainsky) Waksman and Curtis, belongs to Group I Ørskov.

The colonies on agar are glossy, cream coloured and spreading. On potato the growth is abundant, wrinkled, cream coloured, and with a greenish tinge. The aerial mycelium is white, and the spore-bearing hyphae occasionally show short spirals. The conidia are spherical to oval, measuring  $1.1$  to  $1.4\ \mu$  by  $1.2$  to  $1.8\ \mu$ . Gelatine is liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces aureus*, Waksman and Curtis, belongs to Group I Ørskov.

The greyish coloured colonies on agar are restricted in their development. The growth on potato is abundant, wrinkled and brown, becoming black on ageing. A soluble brown pigment is produced. The aerial mycelium is grey to cinnamon drab. The spore-bearing hyphae form numerous spirals. The spores are spherical to oval, measuring  $0.6$  to  $1\ \mu$  by  $0.8$  to  $1.4\ \mu$ . Gelatine is slowly liquefied, starch hydrolysed. Grown in milk a black ring is produced at the surface of the medium.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces exfoliatus*, Waksman and Curtis, belongs to Group I Ørskov.

The colonies on agar, which often do not develop well on the surface, are cream coloured. On potato the growth is somewhat wrinkled, grey, later darkening to brown. The aerial mycelium is white, the hyphae bearing the spores have a tendency to spiral formation. The oval spores measure  $1.0$  to  $1.5\ \mu$  by  $1.2$  to  $1.8\ \mu$ . Gelatine is liquefied, starch hydrolysed, and milk slowly peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces fradii*, Waksman and Curtis, belongs to Group I Ørskov.

The colonies on agar are yellowish, becoming orange-yellow on ageing. On potato the growth is orange coloured. The aerial mycelium is pink and covers the whole surface of the growth. No spirals are formed on the spore-bearing hyphae. The spores are oval to rod-shaped, measuring  $0.5$  to  $0.7\ \mu$  by  $1.25\ \mu$ . Gelatine is liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces lipmanii*, Waksman and Curtis, belongs to Group I Ørskov.

It forms yellow, glossy, radially wrinkled colonies on agar. On potato the abundant growth is wrinkled and cream coloured. The aerial mycelium is white, turning grey. The spores are oval and measure  $0.8$  to  $1.1\ \mu$  by  $1.0$  to  $1.5\ \mu$ . Gelatine is liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces violaceus-caesaris*, Waksman and Curtis, belongs to Group I Ørskov.

It forms thin, cream-coloured colonies on agar; on potato a wrinkled, cream-coloured growth, which turns yellowish on ageing. The aerial mycelium is white. The spore-bearing hyphae occasionally form spirals. The spores are oval to elongated; no measurements are given. The species produces a soluble purple

pigment. Gelatine is slowly liquefied, starch hydrolysed, and milk slowly peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces violaceus-ruber*, Waksman and Curtis, belongs to Group I Ørskov.

The colonies on agar are white, becoming red with white margin. Growth on potato is scarce, folded and brown. The aerial mycelium is white to mouse grey. The spore-bearing hyphae form dextrorse spirals. The spores are oval to rod-shaped and measure 0.7 to 1.0  $\mu$  by 0.8 to 1.5  $\mu$ . A soluble blue pigment is secreted. Gelatine is slowly liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

*Actinomyces viridochromogenus*, (Krainsky) Waksman and Curtis, belongs to Group I Ørskov.

The colonies on agar are large, grey with a greenish tinge. On potato the growth is abundant and grey-brown. The aerial mycelium is white, later becoming greenish. The spore-bearing hyphae form numerous open spirals. The spores are spherical to oval, 1.25 to 1.5  $\mu$ . A soluble brown pigment is formed. Gelatine is slowly liquefied, starch hydrolysed, and milk peptonized.

Habitat: soil. Aerobic and mesophilic.

In addition to the above types Brussoff<sup>10</sup> has isolated a cellulose-decomposing actinomycete, *Actinomyces cloacae*, from the Aachen sewage disposal system. The following are the characteristics of this organism. It belongs to Group I Ørskov, and forms spherical spores.

The aerial hyphae are straight and are formed in rings over the surface of the growth. The spores are white, later becoming sepia coloured, especially when grown on potato. Gelatine is not liquefied. Starch is probably hydrolysed and its structure destroyed, since the colonies grown on potato slowly sink into this substratum. Its optimum temperature for growth is stated to be between 30° and 33° C.

In none of the cases mentioned has the decomposition of the cellulose been followed from a biochemical point of view, and the decomposition products formed by the action of the actinomycetes are unknown.

As regards the action of actinomycetes on hemicelluloses, pectin, and gums, practically no information is available beyond a suggestion by Krainsky that the appearance of

a clear zone round a colony growing on a cellulose agar plate, prepared as recommended by McBeth and Scales, might have been due to the decomposition of the gelose of the agar rather than to the destruction of the cellulose.

The existence of plant-pathogenic forms responsible for the production of potato scab is an indication that pectin may be decomposed. Unpublished experiments by the writers entirely confirm this and have shown that gums and pectin are highly suitable sources of carbohydrates for a large number of actinomycetes.

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## CHAPTER V\*

### THE EUMYCETES. GROUP A.

ATTEMPTS have been made from time to time to determine the relative importance of bacteria, actinomycetes, and fungi in the natural decomposition of vegetable matter. Thus Ramann, Remelé, Shellhorn, and Krause<sup>1</sup>, who determined the ratio of bacteria to fungi in various forest soils, found as a rule more fungi than bacteria in the loose soil underneath the layer of dead leaves and humus. Faelli<sup>2</sup>, on the other hand, in his examination of the soil of the agro Romano found five times as many bacteria as fungi, while Heukelekian<sup>3</sup> arrived at the conclusion that fungi were far more important for the breakdown of cellulose in the soil than either bacteria or actinomycetes.

Though of considerable interest, none of these conclusions is very convincing, since no method exists by which it is possible to establish the quantitative distribution of cellulose-decomposing bacteria in soil.

Whether or not the bacteria may finally be shown to be the more active types, the fact remains that the fungi are very active in the natural decomposition of vegetable debris. Their less specialized food requirements have probably something to do with this. One single species will thus frequently be capable of decomposing gums, pectin, hemicelluloses, and cellulose, and often lignin in addition, while a species of a bacterium is usually able to deal with one of these substances only.

The great adaptability of the fungi will always assure them of an ample supply of carbohydrates in vegetable debris and will thus greatly favour their development. Even in wood

\* The references given in Chapters V and VI (The Eumycetes, Groups A and B) are numbered consecutively as though for a single chapter. They will be found at the end of Chapter VI on pp. 156-8.

there will be an abundance of food materials available, and this is no doubt the reason why fungi take so conspicuous a part in the destruction of this type of vegetable matter. The presence of starch and sugar, which occur in all woods during certain seasons, may be a contributory factor to the ease with which the fungi gain access to their hosts.

A very instructive picture of the action of fungi on wood and other vegetable tissues is given by Schellenberg<sup>4</sup> in a paper of 1922. He distinguishes three stages in the breakdown of plant tissues by filamentous fungi. The first, for which parasitic forms such as the *Uredineae* and the *Ustilagineae* are responsible, results in the destruction of sugar, starch, and dextrins. The second, in which saprophytic fungi such as *Mucor*, *Penicillium*, and *Aspergillus* operate, results in the decomposition of sugar, starch, dextrins, hemicelluloses, and pectin. The third stage, involving the destruction of all of the above carbohydrates, in addition to the cellulose and frequently the lignin, is brought about by the activity of the wood-destroying fungi proper, including both parasitic and saprophytic forms such as the *Polyporaceae* and *Agaricaceae*. As in the case of the fungi which decompose sugars, starch, pectin, and hemicelluloses, these fungi start their attack by the destruction of the readily assimilated carbohydrates, and proceed to the decomposition of the cellulose and lignin only after the supply of sugars and pectin has been more or less exhausted.

The account given in the following pages of the more significant of those saprophytic fungi which have been shown to participate in the natural decay of plant tissues has been compiled from a similar physiological standpoint to that of Schellenberg. The various types which will be discussed are divided into two main groups, those which decompose pectin and hemicelluloses, and those which, in addition, are able to break down cellulose and lignin. As no information of any importance appears to be available of a fungal flora specific to gums, this substance has not been considered in this chapter. Among the higher saprophytic fungi attention has been concentrated on the wood-destroying forms. The list given of these should not, however, be regarded as complete. The subject of wood decay

is still in far too active a state of development, and too many of the responsible fungi are insufficiently described, for it to be possible to give an exhaustive account of all of them. Hitherto, the description of these forms has been confined in a large measure to an account of their fructifications, and they have in consequence become removed from the sphere of microbiological study. More recently, however, there has been a tendency to concentrate on other biological characters, particularly on the morphology and physiology of their mycelium, as grown in the laboratory on artificial media. Thereby, not only have they been brought into line with other micro-organisms, but the circumscription of the various species has been made more definite. The work in this direction will be discussed in greater detail in Chapter XII.

Except where otherwise stated, all the descriptions of the fungi discussed here have been taken from Rabenhorst<sup>5</sup>.

#### GROUP A. *Eumycetes* which decompose pectin and hemicelluloses.

Sub-class: PHYCOMYCETES.

Order: ZYGOMYCETALES. Sub-order: MUCORINEAE.

Family: *Mucoraceae*.

*Mucor globosus*, Fischer.

This species was found by Schellenberg<sup>6</sup> to decompose the hemicelluloses of *Molinia caerulea*, *Lupinus hirsutus*, and *Impatiens balsamina*. It had no action on the hemicelluloses of *Ruscus aculeatus* or of *Phoenix dactylifera*.

The fungus has slightly drooping hyaline sporangiophores which are kept erect by mutual support. They measure from 1 to 3 cms. in height and 6 to 10  $\mu$  in diameter. They are widely branched in sympodial cymes, the branches terminating in sporangia, beneath each of which a septum is formed. The sporangia are spherical, measuring from 75 to 120  $\mu$  in diameter. When ripe they are greyish-brown to dark brown in colour. After resolution of the wall of the sporangium, a basal collar remains. The columella is generally pear-shaped, and as a rule 40  $\mu$  in height and from 6 to 32  $\mu$  in diameter. Its surface is smooth and greyish in colour. The spores, measuring from 4 to 8  $\mu$ , are spherical and of a blackish colour when observed collectively.

*Mucor hiemalis*, Wehmer.

This was identified by Behrens<sup>7</sup> with the dew-retting of flax and hemp, though Ruschmann<sup>8</sup> regards it as of minor importance in this respect.

The colonies are silky, white, and occasionally yellowish-brown. The hyphae are irregular, 7 to 14  $\mu$  or even 30  $\mu$  in diameter. The sporangiophores are simple, occasionally branched, erect, but collapsing later. The sporangia are globose, yellow or grey, 55  $\mu$  in diameter, hyaline and shiny. The columella is globose to ovoid in shape, hyaline, 28 to 48  $\mu$  in diameter. The spores are numerous, thin-walled, and variable in size and shape, averaging 7 by 3.2  $\mu$ . Zygospores have not been observed (Saccardo<sup>9</sup>).

*Mucor neglectus*, Vuillemin.

This species decomposes the hemicelluloses of *Lupinus hirsutus*, *Lupinus albus*, and *Impatiens balsamina* (Schellenberg<sup>6</sup>).

It has erect and sympodially branched sporangiophores, occurring as a matted growth. The branches terminate in sporangia which have no distinctive characteristics. The spherical spores measure only 3  $\mu$  in diameter. Azygospores, but not zygospores, have been reported. They measure 54  $\mu$  in diameter, and their outer membrane, which is thickened in places, is yellow-brown to dark brown in colour.

*Mucor piriformis*, Fischer.

This species decomposes the hemicelluloses of *Lupinus hirsutus* and of *Impatiens balsamina*, but not those of *Molinia caerulea* or the other types tested by Schellenberg<sup>6</sup>.

It has erect but slightly drooping sporangiophores occurring in thinly matted areas. They are 2 to 3 cms. in height and 35 to 50  $\mu$  in diameter. They are generally unbranched, but occasionally side branches occur which are usually sterile. The sporangia are spherical and measure 250 to 350  $\mu$  in diameter. When young they are white, but become greenish-grey to black later. The outer wall possesses closely arranged delicate spines. The columella is generally pear-shaped, measuring 200 to 300  $\mu$  in height and 80 to 280  $\mu$  in width. The spores are uniform and ellipsoidal, measuring 5 to 13  $\mu$  in length and 4 to 8  $\mu$  in width.

*Mucor racemosus*, Fresenius.

Syn.: *Pleurocystis Fresenii*, Bonorden.

*Chlamydomucor racemosus*, Brefeld.

For further synonyms see Rabenhorst<sup>5</sup>.

This species attacks the hemicelluloses of *Molinia caerulea* (Schellenberg<sup>9</sup>). It was found by Oudemans and Koning<sup>10</sup> in many of the humus samples examined by them.

It has erect sporangiophores forming yellowish-brown matted layers. The sporangiophores vary in height from 0.5 to 4 cms. and have a diameter of 8 to 20  $\mu$ . They are irregularly branched in racemes, each branch bearing a sporangium. A septum is formed beneath the sporangium. The sporangia are spherical, measuring 20 to 70  $\mu$  in diameter, are drooping or erect, and yellowish in colour. On the bursting of the sporangial wall a basal collar remains. The columella is club-shaped, 17 to 60  $\mu$  in height and 7 to 42  $\mu$  in diameter. The spores are irregular, and may be spherical or elliptical, measuring 5 to 8  $\mu$  in width and 6 to 10  $\mu$  long. They are smooth and yellow. The zygospores are spherical, brown, and measure 70 to 80  $\mu$  in diameter. On their outer membrane yellowish to red-brown warts are formed. The suspensors are much smaller than the zygospores. Azygospores are occasionally observed, and chlamydospores are also found.

This species, though one of the commonest of the genus *Mucor*, is not very well circumscribed.

*Mucor Rouxianus*, Wehmer.

Syn.: *Amylomyces Rouxii*, Calmette.

*Mucor Rouxii*, Wehmer.

This type was isolated from 'Chinese yeast', in which it represents the amycolytic agent. That it is capable of decomposing hemicelluloses was pointed out by Mezzadrola<sup>11</sup>, who used it for the hydrolysis of vegetable ivory, the endosperm of *Phytalephas macrocarpa*.

The fungus forms a slightly elevated downy growth on wort-gelatine. It grows better on a rice medium, where it forms sporangia. The sporangiophores are small, 1 mm. in height, 7 to 14  $\mu$  in diameter, erect or drooping, usually branched, and bear two or more sporangia, all of one type. They have short pedicels, are erect or leaning, and often abnormal. They form a loose mat, which is orange-red on rice. The sporangia are light or yellowish, round, often broader than they are high, 50  $\mu$  in diameter, smooth and translucent. The membrane of the sporangium is colourless and transparent and, after dissolution, leaves a collar at the base of the columella. The columella is free, rounded, slightly flattened (20 by 23  $\mu$  to 28 by 32  $\mu$ ), smooth and colourless. The spores measure 5 by 2.8  $\mu$ , are colourless, smooth, refractive, and have heterogeneous contents, contracted somewhat from the spore wall. Chlamydospores are abundant, small or large, irregular in

form, 12 to 100  $\mu$  in diameter, and yellowish, light brown, or colourless. Their membrane is smooth, colourless, and thickened up to 7  $\mu$ . Zygospores are unknown. Budding cells are present (Lendner<sup>12</sup>).

*Mucor spinosus*, van Tieghem.

Syn.: *Mucor plumbeus*, Bonorden.

*Mucor aspergilloides*, Zopf.

This species was observed by Ruschmann<sup>8</sup> on flax which had undergone dew-retting, and it probably decomposes pectin.

It possesses straight smooth-walled sporangiophores branching monopodially or in sympodial cymes, the branches each terminating in a sporangium. Each sporangiophore has a transverse septum beneath the sporangium. The sporangia, which are spherical, reach a diameter up to 100  $\mu$ , and become dark brown with age. After the resolution of the sporangial wall a basal collar remains. The columella, which is pear-shaped to cylindrical, measures 8 to 65  $\mu$  in width and 25 to 85  $\mu$  in height. Its membrane is coloured grey to brownish. The spores are smooth, spherical, and greyish-brown, and measure 5 to 9  $\mu$  in diameter. The zygospores are spherical and yellowish-brown in colour. Chlamydospores have been observed.

*Mucor stolonifer*, Ehrenberg.

Syn.: *Ascophora mucedo*, Tode.

*Rhizopus nigricans*, Ehrenberg.

For other synonyms see Rabenhorst<sup>5</sup>.

This species was found by Behrens<sup>7</sup> in large numbers on hemp which had been dew-retted during summer. Ruschmann<sup>8</sup> also observed it on dew-retted hemp. As early as 1890 Kean<sup>13</sup> demonstrated the presence of a pectin-resolving enzyme in this fungus capable of dissolving the middle lamellae of the cells of the sweet potato, *Ipomoea batatas*. Harter and Weimer<sup>14,15</sup> showed this enzyme to be present not only in the mycelium but also in the spores. Schellenberg<sup>6</sup> found the fungus capable of decomposing the hemicelluloses contained in the cotyledons of *Lupinus hirsutus*, *Lupinus albus*, and *Impatiens balsamina*, but not those contained in the seedlings of *Molinia caerulea* or in the seeds of *Phoenix dactylifera*. Schmidt, Peterson, and Fred<sup>16</sup> found that it was capable of decomposing pentosans.

*Mucor stolonifer* owes its name to its manner of growth. The

mycelium spreads as stolons over the surface of the medium. At intervals up to 3 cms. along the creeping white stolons, which later become brown owing to a discoloration of the walls, masses of simple or branched rhizoidal hyphae, also darkening with age, grow down into the substratum. They measure 5 to 16  $\mu$  in diameter and are occasionally septate. From the points opposite these rhizoids groups of three to five unbranched erect sporangiophores arise, measuring 0.5 to 4 mms. in height, and 24 to 42  $\mu$  in diameter. Their smooth membrane becomes brown to dark brown with age. The sporangiophore terminates in an hemispherical columella, 70  $\mu$  broad and 90  $\mu$  in height. The dark brown sporangium measures 250 by 320  $\mu$ . The light grey spores, measuring 6 to 17  $\mu$ , are spherical to oval, and possess a double membrane, the outside of which shows vertical striations. The zygospores are spherical to ellipsoidal, measuring 160 to 220  $\mu$  in diameter, with a leathery dark brown outer membrane, showing hemispherical hollow warts. The inner membrane is thick, colourless, and fills the cavities in the warts of the outer membrane. The suspensors are uneven in size, and almost as broad as the zygospore. Azygospores are sometimes formed, but not chlamydo-spores.

A number of *Rhizopus* species, chiefly parasitic forms, were investigated by Harter and Weimer<sup>17</sup> for pectin-decomposing properties. Among them were the two saprophytic types, *Rhizopus microsporus*, van Tieghem, and *Rhizopus chinensis*, Saito.

*Thamnidium elegans*, Link.

Syn.: *Melidium subterraneum*, Eschweiler.

*Mucor elegans*, Fries.

*Ascophora elegans*, Corda.

*Mucor mucedo*, de Bary et Woronin.

*Ascophora pulchra*, Preuss.

*Thamnidium van Tieghemii*, Berkeley et Broome.

This species decomposes the hemicelluloses of *Lupinus hirsutus*, *Lupinus albus*, and *Impatiens balsamina* (Schellenberg<sup>6</sup>).

The sporangiophores are erect and branched. The sporangium on the main stem is spheroidal, contains many spores, and is borne on a large columella. The solitary or verticillate, usually dichotomous, branches have small spherical sporangia at the ends of branchlets. These sporangia contain 4 to 10, or occasionally 1 to 3, spores. The spores from both types of sporangia are similar, being oval, hyaline, sometimes becoming bluish, and measuring 8 to 10  $\mu$  by 6 to 8  $\mu$ .

## Sub-class: MYCOMYCETES.

Order: ASCOMYCETALES. Sub-order: PLECTASCINEAE.

Family: *Aspergillaceae*.

Little work has been done on the action of *Aspergillus* species on hemicelluloses. The following five species were studied by Schmidt, Peterson, and Fred<sup>16</sup> for their behaviour towards the pentosans of maize and rye straw, which were found by them to become decomposed.

*Aspergillus flavus*, Link.Syn.: *Aspergillus flavescens*, Wreden.*Eurotium aspergillus flavus*, de Bary.*Aspergillus subfuscus*, Johan-Olsen (Přibram<sup>18</sup>).

This species forms yellowish-green to dark brown matted colonies. The vegetative mycelium is greyish. The conidiophores, which are sometimes septate, are 0.4 to 0.7 mm. in height and 7 to 10  $\mu$  in diameter. The vesicle is spherical to club-shaped and 30 to 40  $\mu$  in diameter. The complete fructification measures 85  $\mu$  in diameter. The sterigmata are unbranched and particularly closely set at the summit of the vesicle. They measure 20  $\mu$  in height by 6  $\mu$  in width. The conidiospores are generally spherical and smooth, and 4 to 8  $\mu$  in diameter. The chains of spores break up readily.

Its optimum temperature of growth lies between 28 and 37° C., and it is capable of slowly liquefying gelatine.

*Aspergillus fumigatus*, Fresenius.Syn.: *Aspergillus nigrescens*, Robin.

This species occurs on a variety of hosts, including tobacco and decaying potatoes.

It forms green and later grey to dirty brown colonies. The hyphae of the mycelium measure 2 to 3  $\mu$  in diameter. The delicate, closely set, conidiophores are little differentiated from the mycelium, measuring 5 to 6  $\mu$  in diameter and 0.1 to 0.3 mm. in height. The vesicle measures 10 to 20  $\mu$  across. The complete fructification is 30 to 40  $\mu$  in diameter. The unbranched sterigmata are closely set at the upper part of the vesicle, pointing towards it rather than projecting radially. They are 6 to 15  $\mu$  in length. The smooth spherical to oval conidia measure 2 to 3  $\mu$  in diameter. Its optimum temperature of growth is about 37° C.



*Aspergillus glaucus*, Link.Syn.: *Mucor glaucus*, Link.*Monilia glauca*, Persoon.*Mucor aspergillus*, Bulliard.*Aspergillus repens*, Saccardo.*Eurotium aspergillus glaucus*, de Bary.*Eurotium aspergillus medius*, Meissner.

A very common species appearing on almost all damp objects.

The colonies are light green when young, later becoming darker to greyish-brown. When old the hyaline hyphae often show a yellow to brownish colour. They measure  $3\mu$  in diameter. The conidiophores are erect, unbranched, 1 to 2 mms. high and  $14\mu$  broad. The vesicle is spherical or slightly oval, measuring 30 to  $60\mu$  in diameter. The closely-set unbranched sterigmata radiate uniformly from the whole surface of the vesicle. They are 10 to  $14\mu$  in length and have a diameter of 5 to  $7\mu$ . The spherical or slightly oval conidia, which are formed in long chains, possess a thick, smooth, later sometimes finely papillate, membrane, and measure 7 to  $15\mu$  in diameter, with an average of about  $10\mu$ .

The perithecia of this species are light brown at first, but darken subsequently. They measure 100 to  $200\mu$  in diameter and contain about 20 asci with 5 to 8 smooth ellipsoidal spores, measuring 5 to  $7\mu$  by 8 to  $10\mu$  and possessing a longitudinal furrow.

*Aspergillus niger*, van Tieghem.Syn.: *Sterigmatocystis niger*, van Tieghem.*Sterigmatocystis antacustica*, Crama.*Aspergillus nigricans*, Wreden.*Aspergillus nigricans*, Cooke.*Sceptromyces Opizii*, Corda.*Cephalosporium sceptromyces*, Bonorden.*Stachylidium sceptrum*, Fries.*Botrytis amenticola*, Opiz.*Eurotium aspergillus niger*, de Bary.

This species is responsible for the cork flavour of bottled wine.

It forms very dark brown colonies. The vegetative hyphae measure about  $3\mu$  in diameter. The conidiophores measure  $18\mu$  in diameter and possess an hyaline, smooth, shiny stem 2 mms. in height. The vesicle, which is often rough, is cylindrical, with a diameter of  $80\mu$ . The branched sterigmata radiate uniformly from the whole surface of the vesicle. The primary portion

attains a height of  $26\mu$  and the branches only  $8\mu$ . Their diameter varies between 3 and  $4.5\mu$ . The diameter of the complete fructification may be as much as  $130\mu$ . The conidiospores are spherical, dark in colour, and  $2.5\mu$  in diameter; they are borne in long chains, and when fully ripe are frequently warty.

*Aspergillus oryzae*, Ahlburg.

Syn.: *Eurotium oryzae*, Ahlburg.

*Aspergillus oryzae*, Cohn.

This is an important species used in the East for the preparation of Saké and soya sauce. It secretes an active diastase which is now prepared technically under the name of taka-diaastase. Newcombe<sup>19</sup> states that reserve cellulose (hemicelluloses?) is decomposed by this species.

The young colonies are usually yellowish-green and occasionally brown to brownish-green. With age they become greyish-brown to dark brown. The vegetative hyphae are white to grey, and measure 3 to  $9\mu$  in diameter with an average of 4 to  $5\mu$ . The conidiophores are closely matted, and are 0.8 to 2 mms. in height, with an hyaline erect stem measuring 10 to  $80\mu$  across. The vesicle is spherical, occasionally somewhat club-shaped, and 50 to  $80\mu$  in diameter. The greenish-yellow to yellow or brownish fructification measures 90 to  $120\mu$  in diameter. The slender sterigmata are borne either evenly over the surface of the vesicle or may be congregated towards the summit. They are 10 to  $20\mu$  in diameter. The spherical conidiospores measure 6 to  $7\mu$  in diameter and may be either smooth or papillate. The conidial chains break up readily.

The diastatic enzyme has an optimum temperature of  $50^{\circ}\text{C}.$ , being destroyed between 60 and  $70^{\circ}\text{C}.$  The fungus develops between the temperatures of 8 and  $45^{\circ}\text{C}.$  with an optimum of  $37^{\circ}\text{C}.$  It is capable of liquefying gelatine.

*Aspergillus Wentii*, Wehmer.

This is used by the natives and the Chinese of Java in the preparation of Tas Gu, or bean pulp. The fungus appears spontaneously on boiled soya beans which have been covered with *Hibiscus* leaves. According to Wehmer<sup>20</sup> it softens the cooked beans, probably by dissolving the middle lamellae of the bean tissues.

The mycelium of *Aspergillus Wentii* is at first white, later turning reddish-brown. The conidiophores are conspicuous, 2 to 3 mms. in height, and terminate in vesicles measuring 75 to  $90\mu$

in diameter. The vesicle is covered with slender unbranched sterigmata measuring 4 to  $15\mu$  in length. The spores are spherical or slightly elongated and measure 4 to  $5\mu$  in diameter (Wehmer<sup>20</sup>).

*Penicillium expansum* (Link), Thom.

Syn.: *Coremium glaucum*, Link.

*Floccaria glauca*, Greville.

*Penicillium glaucum*, Link (in part.).

*Coremium vulgare*, Corda (in part.).

Possibly *Penicillium clongatum*, Dierckx.

For other synonyms see Rabenhorst<sup>5</sup>.

This species was tested for its pectin-decomposing properties by Behrens<sup>21</sup> who found them to be positive. It has been found on dew-retted flax. Schellenberg<sup>6</sup> studied three strains of this fungus for decomposition of hemicelluloses and found two of them active and one uncertain. Schmidt, Peterson, and Fredl<sup>16</sup> obtained a decomposition of pentosans by means of a strain of *Penicillium glaucum*.

On ordinary media the colonies of this species are green, becoming grey-green to brown after several weeks, especially after exposure to light. Loose tufts of short coremium-like conidiophores arise in concentric zones from the colonies. They do not exceed 1 to 2 mms. in height, except in old colonies grown on sugar-containing media. The conidiophores may be very short lateral branches of aerial hyphae or may arise singly or in groups to form coremia. The conidiophores possess 1 to 3 main branches bearing verticils supporting numerous sterigmata, which continue to produce great numbers of conidiospores for some weeks, particularly when grown in sugar solution. The sterigmata measure 8 to  $10\mu$  in height and 2 to  $3\mu$  in diameter. The conidiospores are spherical to elliptical, measuring 2 by  $3.3\mu$  to 3 by  $3.4\mu$ . They are green, homogeneous, and not easily detached from the chains in which they are formed.

The species grows readily on all common media and is capable of slowly liquefying gelatine (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Eidamia*, Lindau.

The *Eidamia* species described below are dealt with here rather than under the *Fungi Imperfecti*, since they are related to *Aspergillus*, and the type species *Eidamia acremonioides*, Harz, bears bulbils which, according to Horne and Williamson<sup>23</sup>,

are to be regarded as sterile perithecia. These are absent, however, in the two newer species described below.

*Eidamia viridescens*, Horne et Williamson.

This was shown to decompose pectin with the production of traces of organic acids (Horne and Williamson<sup>23</sup>). The solution in which the pectin had been decomposed was stained green and was capable of reducing Fehling's solution.

The mycelium of this species is hyaline and septate, with branched hyphae measuring 7 to 11  $\mu$  in diameter. The conidiophores are branched and septate with single or grouped flask-shaped sterigmata 1.5 to 3  $\mu$  in diameter and 8 to 10  $\mu$  in length. They bear yellow to green spherical or ellipsoid conidiospores in groups or short chains. They measure 2.5 to 4.5  $\mu$  in diameter and 4 to 5  $\mu$  in length. Hyaline macrospores are borne singly at the tips of lateral branches. They are thick-walled, almost spherical, and measure 8 to 13  $\mu$  in diameter (Horne and Williamson).

*Eidamia catenulata*, Horne et Williamson.

This species also decomposes pectin, though to a lesser extent than *Eidamia viridescens*. Like the latter, it produces organic acids, but the solution of the decomposed pectin is not stained green and does not reduce Fehling's solution.

*Eidamia catenulata* has an hyaline, septate, and branched mycelium, 3 to 6  $\mu$  in diameter. The conidiophores are erect, septate, and sometimes branched. The slender sterigmata, somewhat swollen at the base, measure 1 to 2.5  $\mu$  by 8 to 16  $\mu$ . They occur singly or in groups on the unbranched hyphae or at the tips of the branched conidiophores. They may also be found at the tips of short swollen branches. The conidia are formed in chains of about an hundred. They are slightly or widely elliptical, acute at both ends, and measure 2 to 3.5  $\mu$  by 4 to 7  $\mu$ . They are yellow in colour. Hyaline macrospores occur singly or in pairs at the tips of short branches; they are almost spherical, measuring 7.5 by 8.5  $\mu$ , or pyriform, measuring 14  $\mu$  by 10 to 18  $\mu$  (Horne and Williamson).

The type species, *Eidamia acremonioides*, Harz, has been observed on dead plant material (Rabenhorst<sup>5</sup>), and was found to develop well on seasoned wood of pine, chestnut, and oak (Horne and Williamson). Nevertheless, the last-named authors found it unable to decompose pectin or cellulose.

## Sub-order: PYRENOHYCETIINEAE.

Family: *Hypocreaceae*.*Nectria cinnabarina*, Tode.Syn.: *Sphaeria cinnabarina*, Tode.*Sphaeria decolorans*, Persoon.*Cucurbitaria cinnabarina*, Fries.The conidial form of this species was previously known as *Tubercularia vulgaris*, Tode.

*Nectria cinnabarina* was studied by Schellenberg<sup>6</sup> for its action on hemicelluloses and was found by him to be capable of breaking down those of *Molinia caerulea* and *Lupinus hirsutus*.

The conidial form produces cinnabar-red pustules on decaying wood. After the production of conidiospores, the pustules serve as stromata for the perithecia. The asci are cylindrical to club-shaped, sessile or tapering to form a small stem. They measure 60 to 90  $\mu$  in length and 9 to 12  $\mu$  in width. The paraphyses are thick, branched, and generally club-shaped. The asci contain eight spores, in one or two rows. These are hyaline, straight or slightly curved, with rounded ends. They contain two cells and measure 4 to 7  $\mu$  in diameter and 12 to 20  $\mu$  in length.

Although the species is normally saprophytic, it is able to invade living trees by first getting a footing on any dead tissues present. Other species of this genus may possibly have a similar action on pectin or hemicelluloses, but no positive evidence for this appears to be available.

Family: *Xylariaceae*.*Xylaria hypoxylon*, Linnaeus.Syn.: *Clavaria hypoxylon*, Linnaeus.*Clavaria hirta*, Batsch.*Clavaria cornuta*, Bulliard.*Valsa digitata*, Scopoli.*Sphaeria cornuta*, Hoffmann.*Sphaeria digitata*, Bolton.*Sphaeria hypoxylon*, Persoon.*Sphaeria ramosa*, Dixon.*Xylaria digitata*, Schrank.*Xylaria hypoxylon*, Greville.*Hypoxylon vulgare*, Link.

This species was found by Gatin and Molliard<sup>24</sup> to be capable of decomposing pectin and various hemicelluloses, including vegetable ivory. It is one of 200 species of this genus and is frequently found on old tree stumps. It has been shown by Moliseh<sup>25</sup> to be capable of rendering decaying wood phosphorescent.

The stromata are erect, branched or simple, generally flattened and sometimes cylindrical, 3 to 8 cms. high and black in colour. They are usually differentiated into a stem and a clearly defined fertile region, the latter being generally cylindrical. It is covered by the egg-shaped, closely-set, black perithecia. The asci are cylindrical, are borne on a long stem, and measure  $80\mu$  long and 7 to  $8\mu$  in diameter. They contain eight black spindle-shaped ascospores which have rounded ends and measure 5 to  $6\mu$  in width and 12 to  $16\mu$  in length.

Though this is the only species of the genus on which definite information is available as to an action on hemicelluloses, it is highly probable that many other species of this genus possess similar properties.

Sub-order: DISCOMYCETIINEAE.

Family: *Helotiaceae*.

*Sclerotinia Fuckeliana*, de Bary.

Syn.: *Botrytis cinerea*, Persoon.

For other synonyms see Rabenhorst<sup>5</sup>.

The conidial stage of this fungus was long known as *Botrytis cinerea*, Persoon. It is capable of growing both parasitically and saprophytically, and is extremely common on vegetable matter. It is probably an aggregate of many different minor species. It is capable of dissolving the middle lamellae of the attacked plant tissues (Brown<sup>26</sup> and Blackman and Welsford<sup>27</sup>), and according to Schellenberg<sup>6</sup> decomposes the hemicelluloses of *Lupinus hirsutus*, *Lupinus albus*, and *Impatiens balsamina*.

The mycelial growth forms extensive layers of grey-green, dark olive-green to blackish-brown colour, which appear dusty owing to the presence of the conidia. The conidiophores are erect, septate, and generally unbranched, measuring 11 to  $23\mu$  in diameter. They are coloured blackish-brown at the base and

along the lower part. At the top they are divided into three or more hemispherical protuberances on which the conidia arise, each from a minute projection. By the continued growth of the tip of the conidiophore, the projections are forced sideways. The conidia are usually ovoid, sometimes almost spherical, and possess a small point at the end by which they were connected to the conidiophores. They measure 6.5 to 10  $\mu$  in width and 9 to 12  $\mu$ , occasionally 15  $\mu$ , in length, and may be of a brownish colour. The complete fructification resembles bunches of grapes.

*Botrytis vulgaris*, Fries.

This is probably identical with *Botrytis cinerea*, Persoon, and it is given by Rabenhorst<sup>5</sup> as a synonym for that species. But it should be noted that its action on hemicelluloses differs from that of *Botrytis cinerea* (Schellenberg<sup>6</sup>), the latter being unable to decompose the hemicelluloses of *Molinia caerulea*. In addition to these, *Botrytis vulgaris* decomposes the hemicelluloses of *Lupinus hirsutus*, *Lupinus albus*, and *Impatiens balsamina*. It also attacks fruit in storage (Behrens<sup>21</sup>).

*Sclerotinia sclerotiorum*, Brefeld.

Syn.: *Sclerotinia Libertiana*, Fuckel.

*Peziza sclerotiorum*, Libert.

*Phiala sclerotiorum*, Gillet.

*Hymenoscypha sclerotiorum*, Phillips.

*Peziza sclerotii*, Fuckel.

*Peziza Kauffmanniana*, Tichomerow.

*Rutstroemia homocarpa*, Karsten.

*Peziza postuma*, Berkeley et Wilson.

De Bary<sup>28</sup> found that this species destroyed the middle lamellae of the cells of its host and thus was capable of decomposing pectin. Whether it decomposes hemicelluloses has not yet been established.

*Sclerotinia sclerotiorum* and its related species form hard black sclerotia. These bodies, which in this species attain a diameter of more than 8 mms., consist of dense masses of closely-matted mycelium, constituting a very resistant resting form of the fungus. The mycelium itself is white, and the sclerotia arise in the first place as white spherical cushions; these gradually harden and become black on the surface, but remain white internally. From one sclerotium several long slender stems may arise bearing apothecia. These are closed when young, but later open out to

become funnel-shaped, and finally become almost flat. They are light brownish-yellow in colour, and measure about 1 cm. in diameter. Within, they bear long cylindrical asci interspersed with filamentous paraphyses. Each ascus contains eight hyaline spores which are elliptical, measuring 9 to 13  $\mu$  by 4 to 6  $\mu$ . Unlike some of the related species, this fungus has no '*Botrytis*' stage.

(The above description is compiled from various sources.)

#### Order: BASIDIOMYCETALES.

The biochemical reactions of these fungi have only been studied in comparatively few cases, but, as Schellenberg<sup>4</sup> remarks, there is every reason to believe that they decompose not only cellulose and lignocellulose, but also pectin and hemicelluloses. A detailed description of some of these types is given later under the cellulose-decomposing fungi.

#### FUNGI IMPERFECTI.

##### Order: HYPHOMYCETALES.

This appears to be the only order of the *Fungi Imperfecti* containing saprophytic species which have actually been shown to decompose pectin or hemicelluloses.

Family: *Mucedinaceae*. Sub-family: *Hyalosporae*.

Subdivision: *Oosporae*.

*Monilia sitophila*, (Montagne) Saccardo.

This fungus is utilized by the natives of Java for the preparation of 'Ontjom' cakes, a food prepared from the nuts of *Arachis hypogaea*. From the information supplied by Went<sup>20</sup> it appears that the fungus is capable of dissolving the pectin of these nuts; whether it also decomposes the hemicelluloses present is not clear. Its behaviour towards cellulose will be discussed later.

Went<sup>20</sup> describes the fungus as forming a much-branched mycelium of septate hyphae on solid media, the diameter of the hyphae depending on the medium. The conidiophores show a well-developed tree-like branching and bear chains of conidia which may be either branched or unbranched, and which are formed by segmentation of a branch of the conidiophore. When



food is abundant the conidia often occur in large groups. The individual conidia may sometimes bud—an occurrence which is regarded by Went as a premature germination. Thereby a new branch or string of conidia arises. The partition walls of the conidiospores are thicker in the centre, and under high magnification are seen to consist, when the conidia reach maturity, of strands of intercellular substance separating the spores. The diameter of the conidia varies between 5 and 14  $\mu$ .

Went states that brown perithecia, measuring 0.1 to 0.2 mms. in diameter, are formed in old cultures in the presence of abundant food-supply. This indicates that the fungus should be taken out of the *Fungi Imperfecti* and placed in the *Ascomycetales*.

Sub-family: *Hyalodidymae*.

*Trichothecium roseum*, Link.

Syn.: *Puccinia rosea*, Corda.

*Dactylium roseum*, Berkeley.

*Cephalothecium roseum*, Corda.

*Cephalothecium candidum*, Bonorden.

This species is a widely distributed saprophyte on dead and decaying vegetable matter. It occurs parasitically on apples. Schellenberg<sup>6</sup> found it to be capable of decomposing all the hemicelluloses tested by him, including that of the seed of *Phoenix dactylifera*. Heller<sup>30</sup>, however, could not observe any action either on this or on the hemicellulose of the endosperm of *Phytalephas macrocarpa* (vegetable ivory). That it must be capable of decomposing pectin is clear from its destruction of apple tissues.

The colonies of this species form a powdery covering on the surface of the substratum, and are white at first, but later become rose-red. Like the hyphae of the mycelium, the conidiophores are septate and generally unbranched, with little or no terminal swelling. The conidia arise singly or in clusters from the tip of the conidiophore, and are pear-shaped, with a constriction at the region where a septum divides the spore into two cells. They are at first hyaline, but later rose-coloured, and measure 8 to 10  $\mu$  in width by 12 to 18  $\mu$  in length.

Other species of this genus, such as *Trichothecium domesticum*, Fries, and *Trichothecium sublutescens*, Peck, have been found on dead plant tissues, and may possibly decompose pectin and hemicelluloses. Definite proof of this, however, appears to be lacking.

Family: *Dematiaceae*. Sub-family: *Phaeodidymae*.

Subdivision: *Cladosporieae*.

*Cladosporium herbarum*, Persoon.

Syn.: *Dematium herbarum*, Persoon.

*Acladium herbarum*, Link.

*Cladosporium herbarum*, Link.

*Byssus caespitosa*, Rothert.

*Dematium brassicae*, Persoon.

*Dematium conicum*, Schumann.

*Penicillium cladosporoides*, Saccardo.

*Hormodendron cladosporoides*, Fresenius.

Janczewski<sup>31</sup> considered this to be the conidial stage of an ascomycete, *Mycosphaerella Tulasnei*, but his view is not supported by other authors. Many writers, e. g. Saccardo<sup>9</sup>, Laurent<sup>32</sup>, Massee<sup>33</sup>, Frank<sup>34</sup>, Costantin<sup>35</sup>, Arnaud<sup>36</sup>, have attempted to establish a relationship between this fungus and *Dematium pullulans*, de Bary. It appears certain, however, from the researches of Schostakowitsch<sup>37</sup>, Berlèse<sup>38</sup>, Brooks and Hansford<sup>39</sup>, and Hoggan<sup>40</sup>, that there is no connexion between *Cladosporium herbarum* and *Dematium pullulans*.

*Cladosporium herbarum* is regarded by Ruschmann<sup>8</sup> as the chief dew-retting micro-organism of flax and hemp. Its action on hemicelluloses was studied by Schellenberg<sup>6</sup> who found it to be capable of decomposing the hemicelluloses of *Molinia caerulea*, *Lupinus hirsutus*, and *Lupinus albus*. Brooks and Hansford<sup>39</sup> found it producing black spots on *Laminaria digitata*, where it may have been active as an hemicellulose-decomposer.

The mycelium of this species forms a velvety yellowish-green to greenish-black covering on the medium on which it develops. The conidiophores are erect, septate, sparingly branched, olive-green to brown in colour, and measure 5 to 10  $\mu$  in diameter. They vary considerably in height, reaching as much as 0.3 mms. The conidiospores are oval and arise at the sides of the conidiophores as groups of budding yeast-like cells. They vary in shape, are oblong or ovoid, and unicellular when young, becoming cylindrical or ellipsoid later, and then consist of two to five cells.

Brooks and Hansford<sup>39</sup> give the following more detailed description of the formation of the conidiospores: The conidia originate with the cutting-off of the tip of the conidiophore by a transverse wall. This forms the first conidium, and from this a second conidium is formed by a process of budding, each conidium of

a chain arising as a bud on that immediately behind. The youngest conidium is, therefore, at the distal end of the chain, the oldest being next to the conidiophore. The cell of the conidiophore immediately below the first conidium usually grows out to form a second conidium from which another chain of conidia buds out. The young conidium is cut off from its parent by a transverse membrane which thickens at both ends of the conidium as the latter grows. When the conidia separate, they do so by fission along the line of the membrane. For this reason many of the conidia are lemon-shaped.

Family: *Tuberculariaceae*. Sub-family: *Tuberculariaceae mucedineae*.

Subdivision: *Phragmosporae*.

*Fusarium* species.

The genus *Fusarium* undoubtedly contains a number of species which must be able to decompose both pectin and hemicelluloses, since they are frequently found on decaying vegetable tissues, including cellulose fibres and fabrics. Unfortunately these types are described in the literature under their generic name only, and an enumeration of the various saprophytic species associated with the decomposition of pectin and hemicelluloses is therefore not possible.

## CHAPTER VI\*

### THE EUMYCETES. GROUP B.

GROUP B. **Eumycetes decomposing cellulose and lignin.**

Order: ASCOMYCETALES. Sub-order: PLECTASCIINEAE.

Family: *Gymnoascaceae*.

*Myxotrichum chartarum*, Kunze.

This species was occasionally found by Sée<sup>41</sup> on mildewed paper. It produces a pigment which stains the attacked paper deep brown (see Table II, p. 291).

In liquid cultures the early stages of the mycelial growth form a network of snow-white filaments spreading from the point of inoculation. At this stage the growth resembles that of the genus *Chaetomium*. Later, the mycelium forms small circular, slightly convex, masses floating on the liquid. These gradually spread and cover the surface with a thin somewhat gelatinous veil, dotted with small black spots visible to the naked eye. These are the ascigerous masses, which are formed in about 6 weeks, provided that development occurs at a temperature of not less than 20° C. When mature the fungus consists of (1) a central mass of asci, (2) a network of black filaments, and (3) large crozier-shaped hyphae radiating outwards. The central mass of asci is yellowish in colour. The walls of the asci gelatinize before ripening. The asci represent the swollen ends of extremely fine hyphae. When ripe they measure 5 to 6  $\mu$  by 6 to 8  $\mu$  and contain 8 ovoid spores measuring 2.5 to 3  $\mu$  by 4.5  $\mu$ . These are almost colourless or very pale yellow. On resolution of the cell walls of the asci, the 8 spores adhere together and remain in place. The deep brown to black network consists of di- or trichotomously branched hyphae, the whole mass attaining a diameter of 500 to 700  $\mu$ . The crozier-like hairs measure 2.6  $\mu$  at the base and 150  $\mu$  in length. A second crozier sometimes arises from the curved portion of the first. The coil terminates in a point (Sée<sup>41</sup>).

\* The references given in Chapters V and VI (The Eumycetes, Groups A and B) are numbered consecutively as though for a single chapter. They will be found at the end of Chapter VI on pp. 156-8.

*Eidamella spinosa*, Matruchot et Dassonville.

This was found by Sée<sup>41</sup> on mildewed paper. It stains the attacked paper dark brown (see Table II, p. 291).

The conceptacle is tufted with a covering of thick-walled, much-branched hyphae which turn black. At the distal end they are hyaline and bear at the base 1 to 5 spirally convolute branches. The numerous asci are ovoid, 6 to 7  $\mu$  by 3 to 4  $\mu$ , and bear 8 ovoid to fusiform hyaline spores.

#### Family: *Aspergillaceae*.

The genus *Aspergillus* is often referred to in the literature as a cellulose decomposer without details being given of the species studied. The most interesting of these references is that of Ellenberger and his collaborators<sup>42</sup>, who report that a species of *Aspergillus* termed *Aspergillus cellulosa* occurs widely distributed in the alimentary tract of man and animals. It is stated to decompose cellulose at the rate of about 59 per cent. of the amount present in fifty days. That it should have any bearing on the destruction of cellulose in the intestine, as these authors consider, is most problematical in view of its slow action. In 1919 Hopffe<sup>43</sup> gave a fairly detailed description of this fungus. Reference to this will be found on pp. 98-9.

The following species of *Aspergillus* have been shown to be capable of attacking cellulose.

#### *Aspergillus brunneofuscus*, Sée.

This was found by Sée<sup>41</sup> to produce mildew on paper and was regarded by him as a typical paper-destroying type (see Table II, p. 291).

The growth of this species, which develops rapidly in pure culture, is brown to almost black. The base of the conidiophores is septate—a rare occurrence in *Aspergillus* species. They are sometimes branched and measure 10 to 15  $\mu$  by 150  $\mu$ . The vesicle is ovoid or almost spherical and 35 to 40  $\mu$  in diameter. The unbranched skittle-shaped sterigmata are inserted singly on the upper part of the vesicle and measure 6 to 8  $\mu$  by 20 to 25  $\mu$ . The conidia may be spherical or more often ovoid and measure 8 to 15  $\mu$  in diameter, occasionally as much as 18  $\mu$ . Their surface is papillate. The whole fructification has a diameter of about 75  $\mu$ .

The species secretes a deep garnet-brown pigment sufficient to colour the whole medium in which the fungus is grown. Perithecia are formed (Sée <sup>41</sup>).

*Aspergillus clavatus*, Desmazières.

This species was shown by Scales <sup>44</sup> to decompose the cellulose of filter paper in the presence of ammonium sulphate.

The growth is usually green with a tinge of blue-grey. On ageing it becomes discoloured. The hyphae of the mycelium measure 2 to 3  $\mu$  in diameter. The conidiophores are erect, 1 to 2 mms. in height and 15 to 20  $\mu$  in diameter, terminating in an elongated vesicle of the dimensions 35 by 150  $\mu$ . The whole fructification reaches a size of 70 to 120  $\mu$  by 150 to 250  $\mu$ . When older it is generally less elongated. The sterigmata are unbranched, delicate and skittle-shaped, measuring 2.5 to 3  $\mu$  by 7 to 8  $\mu$ . The spores, which are almost hyaline, are smooth and oval, and are formed in long chains. They measure 3  $\mu$  by 3 to 4.5  $\mu$ .

*Aspergillus flavus*, Link.

This was shown by Scales <sup>44</sup> to decompose cellulose.

A description of its morphological characters is given under Group A (Chapter V).

*Aspergillus fumigatus*, Fresenius.

McBeth and Scales <sup>45</sup> found that this species decomposed pure cellulose in the form of cotton-wool or precipitated filter paper. Lignocellulose in the form of rye straw or cherry wood shavings was not attacked. Its cellulose-decomposing properties were confirmed by Scales <sup>44</sup>, and Heukelekian <sup>3</sup> regards it as one of the most active cellulose decomposers in the soil. Cohn <sup>46</sup> regarded the oxidizing action of the enzymes of this fungus as responsible for the spontaneous heating of germinating barley, an assumption which is not very well substantiated.

The morphological characteristics of the species are described under Group A (Chapter V).

*Aspergillus glaucus*, Saccardo.

As a cause of mildew this species was reported present on paper by Sée <sup>41</sup>, and in cotton goods by Davis, Dreyfus, and Holland <sup>47</sup>, and by Reiners <sup>48</sup>.

A description of its morphology is given under Group A (Chapter V).

*Aspergillus nidulans*, Eidam.

Syn.: *Sterigmatocystis nidulans*, Eidam.

*Aspergillus nidulans*, Winter.

This species was found by Scales<sup>44</sup> to be capable of destroying cellulose in the presence of ammonium sulphate, but not of peptone.

The mycelium when young is orange greenish, but on ageing becomes light green to dirty green. The hyphae measure  $6\mu$  in diameter. The hyaline conidiophores become brownish with age. They attain a height of 0.2 to 0.8 mm., are septate, branched, and thick walled. Their diameter varies between 8 and  $10\mu$ . The vesicle is but little developed and measures 15 to  $20\mu$  in diameter. The hyaline sterigmata are branched, slender when young, and swell on ageing. The primary sterigmata measure  $8\mu$  in length and the secondary only  $7\mu$ . They are borne at the top of the vesicle. The conidiospores are spherical, smooth or echinulate,  $3\mu$  in diameter, and are formed in long chains which often adhere to form large masses.

*Aspergillus niger*, van Tieghem.

Though van Iterson, jr.<sup>49</sup>, considered that the cellulose-decomposing properties of this species were weak, *Aspergillus niger* seems to be extensively met with where cellulose decays. Scales<sup>44</sup> confirms its powers of destroying cellulose and Gerry<sup>50</sup> finds that when growing on wood its hyphae do not perforate the cell walls, but penetrate through natural openings, thus indicating that it does not decompose lignified cellulose. As a cause of mildew it has been observed by Armstead and Harland<sup>51</sup>, Reiners<sup>48</sup>, and Bright, Morris, and Summers<sup>52</sup> on cotton goods. It has been found<sup>53</sup> also on a sample of Manila hemp which showed a large percentage of brittle and discoloured fibres.

A description of *Aspergillus niger* is given under Group A (Chapter V).

*Aspergillus cellulosa*, Hopff.

This type, which is claimed to be closely related to *Aspergillus niger*, is stated to have a vesicle of a diameter of only 4 to  $6\mu$  and

a height of 7 to 11  $\mu$ . The sterigmata are slender, 3 to 5  $\mu$  long. The conidia are formed in short chains of 2 to 3 or possibly more. The spores are grey-brown to black and irregular in shape, occasionally short elliptical, smooth when young, and papillate when older.

*Aspergillus oryzae*, Ahlburg.

Scales<sup>44</sup> found that this species has cellulose-decomposing properties.

Its morphological characters are described under Group A (Chapter V).

*Aspergillus sulphureus*, Desmazières.

This species was occasionally found by Sée<sup>41</sup>. It discolours the paper a yellowish brown to rust colour by the secretion of a pigment (see Table II, p. 291).

Its colonies are light brown when young, but darken with age. The yellowish hyphae measure 2.5 to 3.5  $\mu$  in diameter. The conidiophores are erect and often aggregated into coremia. They show no septation, are hyaline, and measure 4.5 by 280  $\mu$ . The vesicle is inverted, pear-shaped, or spherical. Its maximum measurement is 15 to 30  $\mu$ . The sterigmata are forked. The conidia are more or less spherical, yellowish to yellowish-brown, smooth, and measure 2.5 by 3  $\mu$ . They are borne in chains without intermediate connexions. The long chains arise from the whole surface of the vesicle. The whole fructification measures 50  $\mu$  in diameter (Sée<sup>41</sup>).

*Aspergillus Wentii*, Wehmer.

This species is claimed by Wehmer<sup>20</sup> to decompose cellulose, a view which is supported by Scales<sup>44</sup>, who found it to be capable of destroying cellulose in the presence of ammonium sulphate, but not of peptone.

Its morphological characters were described under Group A (Chapter V).

*Penicillium* species.

As in the case of *Aspergillus*, insufficiently named and described species of *Penicillium* are often referred to in the literature as cellulose decomposers. Thus, Carbone<sup>54</sup> records the action of two species on cotton hairs, which were weakened. Otto<sup>55</sup> mentioned two species decomposing cellulose, while



Davis, Dreyfus, and Holland <sup>47</sup>, Osborn <sup>56</sup>, Levine and Veitch <sup>57</sup>, Armstead and Harland <sup>51</sup>, Reiners <sup>48</sup>, and Bright, Morris, and Summers <sup>52</sup> observed others as mildew on cotton goods. Hauman <sup>53</sup> and Ruschmann <sup>8</sup> remark on the presence of *Penicillium* species on dew-retted flax fibres and on flax and hemp. Heukelekian <sup>3</sup> considers *Penicillium* and the closely related *Citromyces* among the most important cellulose-decomposing fungi of the soil.

The following species have been reported as capable of decomposing cellulose.

*Penicillium africanum*, Doebelt.

This decomposed cellulose in McBeth and Scales's <sup>45</sup> experiments.

The only available morphological data of this species refer to the mycelium. This is greyish-white when young; later, when conidiophores are formed, the colonies become green to dark green and show a yellowish fringe, possibly due to the production of a pigment. The oval conidia measure  $2.4\ \mu$  by  $2.7$  to  $3\ \mu$ .

*Penicillium chrysogenum*, Thom.

Destroyed cellulose in Scales's <sup>44</sup> experiments.

Grown on gelatine and potato agar the species forms a grey-green spreading growth which becomes brownish with age. The margin of the growth is sterile when young. The reverse of the colony shows no discoloration. The conidiophores usually arise separately, sometimes as short branches of aerial hyphae. They generally measure  $4\ \mu$  in diameter and up to  $0.3$  mm. in length. The actual fructifications reach a length of  $100$  to  $200\ \mu$ , with one or two alternate divergent branches, bearing alternate verticillate or twice verticillate branchlets. The sterigmata measure  $2.5$  by  $8\ \mu$ , are verticillate, and bear radiating chains of conidia. The first conidia formed are cylindrical to elliptical, the later more spherical, being  $3$  to  $4\ \mu$  in diameter. They are pale green and contain large vacuoles.

On many media the species produces a golden yellow pigmentation (hence its name). It liquefies gelatine. It is also capable of coagulating milk in the presence of  $0.25$  per cent. calcium chloride (Thom <sup>22</sup>). For further physiological reactions on various media see Thom.

*Penicillium claviforme*, Bainier.

Is regarded as a powerful cellulose decomposer by McBeth and Scales <sup>45</sup> and by Scales <sup>44</sup>.

Grown on lactose gelatine or potato agar the mycelium forms white to grey colonies, the surface of which are composed of loosely arranged floccose hyphae, bearing simple but definitely penicillate fructifications between the bases of white or yellowish branched or unbranched coremia which attain a length of 1 to 2 cms. The coremia are fertile only at the top. The simple conidiophores are sparingly branched, possessing small verticils of sterigmata which measure  $2\mu$  by 9 to  $10\mu$ . The coremial fructifications consist of densely branched interwoven hyphae producing verticils of sterigmata which are crowded into a false hymenium and produce chains of olive-green conidia, adhering to form large masses. They reach a length of 1 to 3 mms. The ellipsoidal conidia measure 3 by  $4.5\mu$ . They are green, homogeneous, and possess a connecting link. The conidial chains do not break up in fluid media.

The species only partially liquefies gelatine, and coagulates milk, in the presence of 0.25 per cent. calcium chloride. It slowly peptonizes milk (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium divaricatum*, Thom.

Though Scales<sup>44</sup> did not find any action on cellulose by this species, Gerry<sup>50</sup> records it as one which is capable of penetrating the cell walls of wood. Its behaviour towards cellulose and wood evidently requires reinvestigation.

Grown on gelatine or bean agar the colonies are brown to hazel, never green. The species spreads widely in the substratum. The aerial growth consists of closely woven fertile hyphae which become powdery in appearance on maturing. The reverse of the colony is not discoloured. The conidiophores are usually short, septate, and mostly creeping. The conidia are formed either terminally or on short branches consisting of separate sterigmata, which are borne as verticils, or as series of verticillate branches and sterigmata irregularly distributed along the fertile hyphae. The cells on which the pointed sterigmata are borne measure  $3\mu$  by 15 to  $20\mu$ . The conidia, which are produced in long chains, are elliptical to fusiform, and measure  $2.5$  to  $3\mu$  by 5 to  $7\mu$ . They are yellowish to brownish, and on germination swell to a size of  $10\mu$ . Two or more tubes are produced by each spore.

The species do not liquefy gelatine. Milk is curdled in the presence of 0.25 per cent. calcium chloride (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium Duclauxii*, Delacroix.

In Scales's<sup>44</sup> experiments this species decomposed cellulose in the presence of ammonium sulphate.

Grown on gelatine the colonies are clear dark green, changing to olive-green when older. The aerial growth consists of crowded conidiophores, usually arising singly from the substratum, but sometimes producing short coremia. Long coremia are formed abundantly on oranges, in milk, on potato, and on all media rich in saccharose. The single conidiophores are very short, reaching a height of only 10 to 50  $\mu$ . When produced on the coremium, they are only found on the uppermost third of it. They show one or two septa and bear a simple conidial fructification or a terminal fructification and in addition a divergent lateral branch with a whorl of conidiiferous cells (sterigmata?). The conidial fructification often reaches a length of 100 to 160  $\mu$ . The conidia are elliptical to fusiform and measure 2 to 2.5  $\mu$  by 3.5 to 4  $\mu$ . They are green and smooth when young, and wrinkled when ripe. Their content is homogeneous.

The species liquefies gelatine and produces a pigment in sugar-containing media which is wine-red by alkaline reaction and bile-yellow in acid media. Milk is curdled in the presence of 0.25 per cent. calcium chloride (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium expansum*, (Link) Thom. .

This is the name given by Thom to a species which includes a part of the former collective species *Penicillium glaucum*, Link, and *Penicillium crustaceum*, Fries. The former is generally recorded as a non-cellulose-decomposing species, but Miyoshi<sup>50</sup> finds that it is capable of perforating membranes containing cellulose, partly through mechanical action and partly through enzymatic activity. Scales<sup>44</sup>, working with a culture of *Penicillium expansum* obtained from Thom, observed decomposition of cellulose by this culture. As a cause of mildew on cotton goods it has been mentioned by Davis, Dreyfus, and Holland<sup>47</sup> and by Reiners<sup>48</sup>. Hauman<sup>58</sup> found it among the micro-organisms present on dew-retted flax fibres.

The morphology of *Penicillium expansum* is given under Group A (Chapter V).

*Penicillium funiculosum*, Thom.

This was found by Scales<sup>44</sup> to decompose cellulose in the presence of ammonium sulphate.

Cultivated on gelatine or potato agar the colonies are deep green and widely spreading. The surface, consisting of pro-

cumbent hyphae, tufts, and ropes of hyphae, appears powdery. The hyphae bear lateral conidiophores. With age the reverse of the colonies becomes red, purple, to almost black, the pigmentation penetrating throughout the medium. The conidiophores, which reach a height of 20 to 100  $\mu$ , usually arise perpendicularly from trailing hyphae, but may arise separately from the substratum. The conidial fructifications reach a length of 125 to 160  $\mu$ , and consist of one or two closely set branches, which bear verticillate branchlets and dense verticils of parallel sterigmata, measuring 2 to 3  $\mu$  by 10 to 14  $\mu$ . The earlier conidia are cylindrical, the later elliptical or fusiform, 2 to 3  $\mu$  by 3 to 4  $\mu$ . They are greenish in colour. The chains break up completely when immersed in liquid.

The species does not liquefy gelatine in two weeks. Milk is curdled in the presence of 0.25 per cent. calcium chloride, and coremia are formed in this medium in old cultures (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium granulatum*, Bainier.

This species decomposed cellulose in Scales's<sup>44</sup> experiments.

Grown on gelatine or potato agar the irregular colonies are yellowish-green to grey or greyish-brown. Their aerial growth consists of massed small coremia, 1 to 3 mms. or less in height, mixed with floccose hyphae and separate conidiophores. The reverse of the colonies is reddish-orange. Characteristic for this species are delicate granular or spinulose hyphae. The conidiophores measure 4 to 4.5  $\mu$  in diameter. The conidial fructifications are usually 100 to 200  $\mu$  in length, once or twice verticillate, and with many sterigmata, measuring 2 to 2.5  $\mu$  by 9  $\mu$ . The conidial chains are long and divergent, the older spores being cylindrical and the younger spherical, measuring about 2.5 to 3  $\mu$  by 3 to 3.5  $\mu$ . They are yellowish-green and adhere closely to one another; they are not, therefore, readily separated on immersion in liquid. Their content is granular.

The species does not liquefy gelatine and milk is not coagulated (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium intricatum*, Thom.

A destruction of cellulose by this species was observed by Scales<sup>44</sup>.

Grown on gelatine the colonies are white, grey to greenish-grey, becoming darker grey on ageing. The older colonies consist of a mass of interwoven hyphae and ropes of hyphae, the latter measuring 1 to 3 mms. in diameter. The reverse of the colonies is more or less sulphur-yellow or even brownish when the fungus is grown on sugar-containing media. The conidiophores are usually formed as branches of aerial hyphae, and reach a height

of 30 to 50  $\mu$ . The length of the conidial fructifications varies between 50 and 140  $\mu$ , the longer occurring in old cultures grown on sugar-containing media. They consist of simple verticils of sterigmata, or of 1 to 3 verticils on divergent branchlets, or of branchlets and sterigmata in the same verticil. The sterigmata measure 2 to 2.5  $\mu$  by 8 to 10  $\mu$ , 4 to 10 being present in each verticil. The chains of conidia are frequently aggregated into loose columns. The conidia are elliptical to spherical, hyaline or pale greenish. They measure 2.5 to 3  $\mu$  in diameter. The cell wall is thin and smooth and the contents granular. The chains do not break up on immersion in liquids.

Gelatine is not liquefied by this species. Milk is slowly curdled in the presence of 0.25 per cent. calcium chloride. Grown on this medium the mycelium is sulphur-yellow (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium lanosum*, Westling.

This species decomposed cellulose in Scales's<sup>44</sup> experiments.

Grown on prune gelatine the colonies are woolly, greyish-green in colour, with a wide sterile margin. The reverse of the colonies is colourless or has a yellowish tinge. The conidiophores are penicillately branched at the top, smooth, 3.4 to 4.6  $\mu$  in diameter and up to 1 mm. in length. The 'metulae' (branches bearing the sterigmata) measure 3 to 4.6  $\mu$  by 12 to 14  $\mu$ , and the sterigmata 2 to 2.7  $\mu$  by 7 to 9.6  $\mu$ . The conidia are small, spherical, uniform, smooth, or slightly rough, and 2.2 to 3  $\mu$  in diameter. Gelatine is slowly and only partly liquefied (Westling<sup>60</sup>).

*Penicillium luteum*, Zukal.

Woltje<sup>61</sup> asserts that this species has no action on filter paper. McBeth and Scales<sup>45</sup> and Scales<sup>44</sup>, on the other hand, record it as a cellulose decomposer.

The growth on sugar-gelatine or potato agar is usually white or grey. On media without sugars it may show greenish areas of conidial fructifications. The aerial growth, particularly of hyphae-producing asci, may show a reddish coloration. Such growth takes several weeks to develop. The colony spreads irregularly on the substratum and has a close floccose appearance. The reverse of the colony is more or less reddish, particularly on sugar-containing media. The scanty conidiophores usually arise as lateral branches of aerial hyphae and measure 3  $\mu$  by 20 to 100  $\mu$ , mostly 30 to 60  $\mu$ . The whole fructification attains a length up to 80  $\mu$ . It frequently possesses a single lateral branch with but two verticils of long acuminate sterigmata, measuring 3 to 4  $\mu$  by 13 to 16  $\mu$ . The conidia are elliptical to fusiform, 2.3 by 2.4  $\mu$  with rather firm walls, and are greenish in colour. On

germination they swell and produce 1 or 2 tubes. Grown on sugar- or starch-containing media they sooner or later produce ascigerous wefts of hyphae, which measure from 0.5 to 2 mms. in diameter. They are spherical and coloured yellowish to red. The asci are red, spherical to fusiform, and measure 7 to 7.8  $\mu$  by 8.8  $\mu$ . The ascospores measure 3.3 by 4.8  $\mu$  and are hyaline to reddish.

The species does not liquefy gelatine, or does so very slowly. The growth on milk is scanty and does not produce curdling (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium notatum*, Westling.

This species gave a positive result when tested for cellulose-destroying powers in Scales's<sup>44</sup> experiments.

Grown on prune gelatine the colonies are floccose, greenish-blue, with a wide sterile margin. The reverse of the colonies is yellowish. The conidiophores are penicillately branched at the top, smooth, 2.8 to 4.6  $\mu$  in diameter and up to 750  $\mu$  long. The 'metulae' (branches bearing the sterigmata) measure 3 to 4.6  $\mu$  by 10.5 to 14  $\mu$ , and the sterigmata 2.2 to 3  $\mu$  by 7 to 8  $\mu$ . The conidia are smooth, almost spherical, and measure 2.6 to 3.2  $\mu$  in diameter. Gelatine is liquefied rapidly (Westling<sup>60</sup>).

*Penicillium pinophilum*, Hedgcock.

Syn.: *Penicillium aurum*, Corda em. Hedgcock.

In McBeth and Scales's<sup>45</sup> experiments this species caused a destruction of cotton and filter paper. It had no action on lignified cellulose. Clark and Scales<sup>62</sup> regard it as one of the most active cellulose-destroying fungi present in soil. Cultures grown artificially on a medium containing ammonium sulphate and peptone reduced filter paper to a soft pulpy mass in about two weeks.

On lactose gelatine or potato agar the colonies are green; on other agar media containing starch or cane-sugar they are yellow-green to bright yellow or orange. Grown on acidified media the aerial hyphae are studded with yellow granules. The reverse of the colonies on such media is deep red. The surface growth consists partly of simple conidiophores, partly of aerial hyphae, and partly of ropes of hyphae, rarely transformed into vertical coremia. Conidiophores are borne as lateral branches on these ropes of hyphae. The conidiophores attain a height of 100 to 200  $\mu$ ; the actual fructifications reach up to 120  $\mu$ . They consist of single verticils of branches measuring 2 to 2.5  $\mu$  by 10 to 16  $\mu$ , and bear whorls of cells 2 to 2.5  $\mu$  by 13 to 15  $\mu$  which taper into acuminate sterigmata bearing parallel chains of conidia. The

elliptical conidia, measuring  $2\mu$  by  $3$  to  $3.6\mu$  are small, pale green to yellowish-green.

The species liquefies gelatine slowly and incompletely. It is responsible for discoloration of commercial timbers. Development is slow on milk, and coagulation tardy, even in the presence of 0.25 per cent. calcium chloride (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium purpurogenum*, Stoll.

Scales<sup>44</sup> finds this species capable of destroying cellulose. Woltje<sup>61</sup>, however, could not find that it had any action on filter paper.

The growth on lactose gelatine and potato agar is grayish-green to brown or olive. On media containing cane-sugar the colour is a somewhat deeper green. The surface growth is closely floccose, almost velvety, and spreads slowly over the substratum, producing a red soluble pigment. Grown on acid media rich in sugar a secondary floccose white mycelium arises, studded with hyphae with yellow granules. The conidiophores measure  $3.5\mu$  by  $100$  to  $300\mu$ , and arise separately, or from portions of hyphae just above the surface of the substratum. The conidial fructification reaches a length of  $50$  to  $100\mu$ , and is composed of one verticil of branches, sometimes with a secondary or partly secondary verticil. They bear whorls of cells  $2.5\mu$  by  $11$  to  $12\mu$ , which narrow abruptly to form sterigmata at their apices. The conidial chains are long and divergent. The elliptical spores measure  $2$  to  $2.5\mu$  by  $3.4$  to  $3.8\mu$ . They are granular and contain one or several highly refractive granules. The conidial chains break up readily when immersed in liquid.

The species slowly liquefies gelatine, and curdles milk in the presence of 0.25 per cent. calcium chloride in 13 days (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium Roqueforti*, Thom.

Syn.: *Penicillium glaucum* some authors, but not Link or Brefeld.

Here again Scales<sup>44</sup> finds a decomposition of cellulose, whereas Woltje<sup>61</sup> records negative results.

Grown on lactose gelatine or potato agar the colonies quickly become green and, on ageing, dirty brown. They spread irregularly over the surface by means of main radiating and branched hyphae with short prostrate aerial loops. The appearance of the colonies is distinctly velvety. The reverse of the colonies is yellowish-white. The conidiophores arise separately and in acropetal succession from the growing parts of the submerged

hyphae. They are septate and reach a height of 200 to 300  $\mu$ . The fructifications measure 90 to 120  $\mu$ , sometimes 30 to 60  $\mu$  by 160  $\mu$ , and at the broadest part usually appear double, owing to the divergence of the lowest branch. The branchlets are irregularly verticillate, bearing crowded verticils of appressed sterigmata measuring 2.5  $\mu$  by 9 to 11  $\mu$ , on which long divergent chains of conidia are borne. The conidiophores are bluish-green, cylindrical to spherical, with a smooth rather firm wall, and measure 4 to 5  $\mu$  in diameter. On germination each produces a single straight tube.

The species does not liquefy gelatine, but softens it somewhat. Milk is curdled in 10 days in the presence of 0.25 per cent. calcium chloride (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium roseum*, Link (?).

This species is reported by McBeth and Scales<sup>45</sup> to be a moderately active cellulose decomposer.

Grown on lactose gelatine or potato agar the colonies are white, shading into pink or salmon colour in the fruiting areas. The surface growth is floccose, consisting of simple hyphae and ropes of hyphae producing in old cultures dense irregular pinkish masses of sclerotia, up to 1 mm. or more in size. The conidiophores are borne as perpendicular branches of aerial hyphae or of the ropes of hyphae, and measure 45 to 125  $\mu$  in height. The conidial fructifications, which attain a length of 140  $\mu$ , are once or twice irregularly or verticillately branched, with spore-bearing cells varying from 2 to 3  $\mu$  by 12  $\mu$ , in the verticils of five or less, to 2 to 3  $\mu$  by 17  $\mu$  when solitary. These sterigmata bear conidia which become aggregated into gelatinous masses. Though the conidia appear colourless when viewed singly, they are pink to rosy in mass. They measure 3 to 5  $\mu$  by 5 to 7  $\mu$ , are slightly apiculate, smooth, and delicately granular within.

The species rapidly liquefies gelatine (Thom<sup>22</sup>).

*Penicillium rugulosum*, Thom.

The cellulose-decomposing properties of this species were observed by McBeth and Scales<sup>45</sup> and by Scales<sup>44</sup>.

Grown on gelatine or bean agar the colonies are yellowish-green, later becoming green to dark green. The surface growth consists of densely crowded conidiophores interspersed at the base with few aerial hyphae. The reverse of the colonies is yellow to orange in patches, particularly when grown on agar or sugar-containing media. The substratum is only discoloured a slight yellow, if at all. The conidiophores, which measure 2.5 to 3  $\mu$  by 100 to 200  $\mu$ , arise separately or as branches of aerial hyphae



immediately above the substratum. The fructifications attain a length of 100 to 150  $\mu$  and consist of appressed verticillate branches measuring 2.5  $\mu$  by 10 to 15  $\mu$ . These bear verticils of spore-bearing cells, or of branchlets, or of spore-bearing cells and branchlets together. The spore-bearing cells measure 2  $\mu$  by 9 to 12  $\mu$ , and are pointed at the end. They bear divergent chains of conidia which are elliptical, measuring 2.5 to 3  $\mu$  by 3.4 to 3.8  $\mu$ , and usually show a swelling at one end. They are green and become wrinkled when ripe. On germination they swell to 5  $\mu$  and produce 1 or 2 tubes.

The species does not liquefy gelatine or does so only partially. Milk is curdled in 9 days in the presence of 0.25 per cent. calcium chloride (Thom<sup>22</sup>). For further physiological data see Thom.

*Penicillium spinulosum*, Thom.

This species was observed by Scales<sup>44</sup> to destroy cellulose in the presence of ammonium sulphate.

Grown on gelatine the colonies are deep green and spread widely over the medium. Their broad margin is sterile when young. The aerial portion consists of conidiophores and scattered aerial hyphae. The reverse of the colonies is not discoloured. The conidiophores measure 3 to 3.5  $\mu$  by 150 to 300  $\mu$  or more. Their apices are enlarged to 5  $\mu$ . Each conidiophore bears a single verticil of spore-bearing cells measuring 2 to 3  $\mu$  by 5.9 to 11  $\mu$ . The conidial fructification forms a close column of spore chains reaching a length of 300 to 500  $\mu$  and a width of 15 to 30  $\mu$ . The conidia are pyriform to spherical, 3 to 3.5  $\mu$  by 3.6 to 4  $\mu$ , very thin-walled, smooth when young, and later delicately wrinkled or spinulose. They are yellowish-green when young, later becoming smoke-grey.

The species liquefies gelatine slowly and curdles milk slowly in the presence of 0.25 per cent. calcium chloride (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Penicillium stoloniferum*, Thom.

Was found by McBeth and Scales<sup>45</sup> to possess marked powers of cellulose destruction.

Grown on gelatine or potato agar the colonies are green to yellowish-green, becoming greenish-grey to grey with age. On sugar-containing media the green coloration is maintained. In young cultures the submerged mycelium appears to arise from the aerial mycelium rather than vice versa. The reverse of the colonies remains uncoloured or is coloured yellowish in patches. The short conidiophores, 100  $\mu$  or less in length, arise as branches from aerial hyphae. At the margin of older colonies longer coni-

diophores arise separately. These attain a length of  $300\ \mu$  or more. The conidial fructifications attain a length of 40 to  $80\ \mu$ , and occasionally as much as  $170\ \mu$ . They are composed of short appressed branches and numerous spore-bearing cells, densely crowded at the base. They bear loosely divergent chains of conidia; sometimes the lowest branch diverges, making the fructification appear double. The spore-bearing cells measure 3 by  $10\ \mu$ . The conidia are spherical to slightly elliptical,  $2.8$  to  $3.4\ \mu$ , smooth and almost hyaline. In masses they are yellowish-green.

The species liquefies gelatine very rapidly and curdles milk in one week in the presence of 0.25 per cent. calcium chloride (Thom<sup>22</sup>). For further physiological reactions see Thom.

*Scopulariopsis repens*, Bainier.

Syn.: *Penicillium Bainieri*, Saccardo.

*Scopulariopsis communis*, Bainier.

*Penicillium brevicaulis*, Saccardo (in part.).

Scales<sup>44</sup> found that this fungus destroyed cellulose in the presence of ammonium sulphate.

The vegetative hyphae are creeping and sparingly septate. The conidiophores are short and twice verticillately branched. The end branchlets (pseudosterigmata) are inverted club-shaped and measure  $14$  to  $28\ \mu$  in length. The conidia are spherical, minutely echinulate, brownish,  $6$  to  $8\ \mu$  in diameter, and are borne in long chains (Saccardo<sup>9</sup>).

#### Sub-order: PYRENOMYCETINEAE.

##### Family: *Sordariaceae*.

*Sordaria humicola*, Oudemans.

This was found by van Iterson, jr.<sup>49</sup>, to possess but little power of destroying cellulose.

No description of this fungus appears in any of Oudemans's published papers, and the name should probably be regarded as *nomen nudum*.

##### Family: *Chaetomiaceae*.

*Chaetomium bostrychodes*, Zopf.

This and other species of *Chaetomium* were frequently found on mildewed paper by Sée<sup>41</sup>. On mildewed cotton

goods unnamed species were found by Osborn<sup>56</sup> and by Bright, Morris, and Summers<sup>52</sup>. *Chaetomium bostrychodes* produces greenish-brown to deep olive-coloured spots on the paper attacked by it (see Table II, p. 291).

The elliptical, fusiform, or almost cylindrical perithecia measure 220 by 340  $\mu$ . They are brown and possess a short papillate hyaline opening. They are surrounded by sparse rhizoids, and at the opening by a tuft of usually regularly spiral and slightly incrustated hairs. The asci are club-shaped, tapering to a short stem, 50  $\mu$  in length and 12  $\mu$  in diameter. The eight spores are elliptical or almost spherical or apiculate, and olive-brown in colour. They measure 5  $\mu$  by 6 to 7  $\mu$ .

*Chaetomium chartarum*, Berkeley.

Syn. : *Ascotricha chartarum*, Berkeley.

This species was frequently found on paper by Sée<sup>41</sup> (Table II, p. 291). Unlike the two other species of *Chaetomium* mentioned here, it does not produce a soluble pigment which diffuses into the attacked paper, but discolours its substratum only through the colour of its brown mycelium. The species was reported as present on mildewed cotton goods by Davis, Dreyfus, and Holland<sup>47</sup>.

The almost spherical perithecia are produced in clusters. Each perithecium has a skittle-shaped neck. The thin wall of the perithecium is fragile and olive-green to brown in colour. The opening is surrounded by a tuft of rigid, diverging, more or less regularly branched, thick-walled, septate, brown hairs. At the ends they are pear-shaped and colourless. Some branches bear clusters of conidia resembling bunches of grapes. The conidia are spherical to elliptical and light brown. The club-shaped asci taper to a short stem and measure 5 to 6  $\mu$  by 70  $\mu$ . They contain a row of eight spores which, observed from the side, are narrowly elliptical, and, from the front, broadly elliptical. In the latter case they measure 5 to 6.5  $\mu$  by 7.5 to 8.5  $\mu$ . They are deep brown to black in colour.

*Chaetomium Kunzeanum*, Zopf.

Syn. : *Chaetomium globosum*, Kunze.

*Chaetomium chartarum*, Ehrenberg.

*Chaetomium feberi*, Corda.

*Chaetomium affine*, Corda.

Van Iterson, jr.<sup>40</sup>, found this species developing on paper, which became slightly decomposed by it. Sée<sup>41</sup> reports it to be a frequent inhabitant of mildewed paper, on which it produces yellowish-brown to brownish-black spots, owing to the secretion of a pigment.

When young the species produces an abundant white mycelium, and occasionally retains this vegetative form when old. Where perithecia are formed they are dark brown to black, ovoid, and measure 250 to 300  $\mu$ . The neck is short, hyaline, and covered with papillae. The brown unbranched hairs surrounding the opening measure 2  $\mu$  in diameter and are coiled at the upper part. The club-shaped asci enclose eight dark brown to black, sharply-pointed spores, measuring 6 to 8  $\mu$  by 11  $\mu$ . Some authors state that chains of conidia are formed (Sée<sup>41</sup>).

Several other species of *Chaetomium* have been recorded present on decaying vegetable tissues and paper, but definite information as to their action on pectin, hemicelluloses, and cellulose is still lacking.

#### Family: *Xylariaceae*.

The fungi of this family are found on old tree-stumps and damp wood.

*Hypoxyylon coccineum*, Bulliard.

Syn.: *Lycoperdon variolosum*, Linnaeus.

*Valsa vagiformis*, Scopoli.

*Sphaeria lycoperdoides*, Weigel.

*Sphaeria rubra*, Willdenow.

*Sphaeria radians*, Tode.

*Sphaeria tuberculosa*, Sowerby.

*Sphaeria fragiformis*, Persoon.

*Sphaeria bicolor*, A. P. de Candolle.

*Sphaeria lateritia*, A. P. de Candolle.

*Stromatosphaeria fragiformis*, Persoon.

This species attacks freshly felled beech-wood, causing a white rot (Tuzson<sup>63</sup>).

Its conidia-forming hymenium may occur in two forms, either as that normal for the *Hypoxyylon* species, i. e. as a powdery covering of the young perithecial stromata, thereby rendered vividly green to yellow or brownish, or on deformed stromata, which remain

sterile. This abnormal type of hymenium occurs on very damp material. The spore-bearing stromata either break through the peridermis of the host or may be formed later on the surface. They are generally almost spherical. When young they are cinnabar-red, and later dark brownish-red on the surface. Their interior is dark brown. They occur singly or in clusters. The egg-shaped perithecia are small and closely set just below the surface of the stroma, giving the latter the appearance of a strawberry. The asci are cylindrical, with a long stem, and possess eight spores surrounded by very long thread-like unbranched paraphyses,  $88\mu$  in length. The diameter of the asci is 6 to  $7\mu$ . The spores are arranged slanting in one row. They are blackish and elliptical, measuring 4 to  $5\mu$  by 10 to  $12\mu$ .

*Xylaria hypoxylon*, Linnaeus.

This was shown by Gatin and Molliard<sup>24</sup> to decompose lignin.

A description of the species is given under Group A (Chapter V).

*Xylaria polymorpha*, Persoon.

Syn.: *Sphaeria polymorpha*, Persoon.

*Valsa clavata*, Scopoli.

*Xylaria clavata*, Schranck.

*Clavaria digitata et hybrida*, Bulliard.

*Sphaeria digitata*, Muller.

*Xylaria polymorpha*, Greville.

Is one of the many known species of *Xylaria* which are common on old tree-stumps. It produces a white rot.

The stromata are formed in clusters of two to six, or more, which are connected at the base. Very occasionally they occur singly. They are erect, sometimes cylindrical, tapering towards the base, sometimes inverted egg-shaped and compressed, frequently forked and sometimes almost spherical. When young they are earthy brown, later becoming blackish. The perithecia occur on the greater part of the stromata, rendering the latter wrinkled. The stem of the stroma is generally short and may occasionally be absent. The perithecia are fairly large, egg-shaped or almost elliptical, with a papillate opening. The asci are cylindrical and possess a long stem. They measure 8 to  $10\mu$  by 140 to  $180\mu$ . They contain a row of eight spores which are elliptical or fusiform, usually pointed at both ends. They may be curved, and are unicellular. They are brown in colour and measure 6 to  $9\mu$  by 20 to  $32\mu$ .

## Sub-order : DISCOMYCETIINEAE.

Family : *Helotiaceae*.*Sclerotinia Fuckeliana*, de Bary.

The conidial stage of this fungus, *Botrytis cinerea*, Persoon, was shown by Kissling<sup>64</sup> to have a solvent action on cellulose. Though Schellenberg<sup>6</sup> could find no action on either cotton or hemp cellulose, the species is now generally regarded as a cellulose-decomposing type.

A description of the species is given under Group A (Chapter V).

*Botrytis vulgaris*, Fries.

Was found by Behrens<sup>21</sup> and by van Itersen, jr.<sup>49</sup>, to be capable of decomposing cellulose. It was isolated from decayed leaves by Oudemans and Koning<sup>10</sup>. In Schellenberg's<sup>6</sup> experiments it could not be shown to act upon cellulose, or at least not on the cellulose of cotton or hemp.

With reference to the identity of this species with *Botrytis cinerea*, Persoon, see under Group A (Chapter V).

Unnamed *Botrytis* species were studied by Otto<sup>55</sup>, Sidbotham<sup>55</sup>, and Bright, Morris, and Summers<sup>52</sup>. In the case of Otto's type, at least, it was shown to be capable of decomposing cellulose.

Family : *Pyronemaceae*.*Pyronema confluens*, Tulasne.

Syn. : *Peziza omphalodes*, Bulliard.

*Pyronema omphalodes*, Fuckel.

*Aleuria omphalodes*, Gillet.

*Peziza confluens*, Persoon.

*Pyronema Marianum*, Carus.

Was found by van Itersen, jr.<sup>49</sup>, to possess moderate cellulose-destroying properties.

A number of apothecia, originally spherical and later expanded, and merging one into another, are formed on an hypothecium composed of hyaline septate hyphae of a diameter of 4 to 5  $\mu$ . The hymenial surface may be flat or raised, and is pink to rose-

red or orange coloured, 0.2 to 2 mms. wide. The asci are cylindrical to rounded, 9 to 10  $\mu$  broad by 120 to 150  $\mu$  in length. They contain a row of eight elliptical unicellular smooth spores, sometimes containing two small oil drops. They measure 6 to 7  $\mu$  by 12 to 15  $\mu$ . The paraphyses are forked at the base, are 3  $\mu$  broad, and colourless or tinged yellowish or red.

Family: *Cenangiaceae*.

*Bulgaria polymorpha*, Wettstein.

Syn.: *Peziza polymorpha*, Oeder.

*Elvellia undecima*, Schaffer.

*Octospora elastica*, Hedwig.

*Peziza brunnea*, Batsch.

*Burcardia turbinata*, Schmiedel.

*Tremella agaricoides*, Retzius.

*Peziza inquinans*, Persoon.

*Bulgaria inquinans*, Fries.

*Ascobolus inquinans*, Noes.

*Peziza nigra*, Bulliard.

*Lycoperdon truncatum*, Linnaeus.

This is a common saprophyte on felled oak and beech. Its development, however, must be rather slow, since it is unable to get a firm footing in the wood if the felled logs are immediately debarked and the wood thus allowed to dry. According to Biffen <sup>66</sup> it decomposes lignin.

The apothecia are developed from the gelatinous fungal tissues, which break through the pericycle of the host. They occur singly or in groups, and are usually egg-shaped, 1 to 4 cms. in height. On opening, the dark brown to black hymenium expands to a width of 1.5 to 4 cms. and shows a transversely wrinkled gelatinous surface in which is embedded an entangled mass of delicate threads. The asci are club-shaped to cylindrical with long stems, often thickened at the top. They measure 9 to 10  $\mu$  by 150 to 200  $\mu$ . When young they contain a row of eight spores, of which four remain hyaline and smaller. The spores are elliptical, somewhat curved, and unicellular, and contain one or sometimes two large oil drops. The four dark brown spores measure 6 to 7  $\mu$  by 12 to 14  $\mu$ . The thread-like paraphyses measure 1  $\mu$  in diameter, but are somewhat wider at the apex, where they are adherent. They are yellow to purplish-brown and often hook-shaped.

Family: *Rhizinaceae*.*Rhizina inflata*, Quélet.Syn.: *Rhizina inflata*, Karsten.*Rhizina undulata*, Fries.*Phallus acaulis*, Batsch.*Helvella acaulis*, Persoon.*Rhizina laevigata*, Fries.*Peziza rhizophora*, Willdenow.*Octospora rhizophora*, Hedwig.

This fungus is sometimes found growing luxuriantly on old heaps of sawdust (Masse<sup>67</sup>), and on burnt soil, peat, &c. It occurs also as a root parasite on young coniferous trees.

The fruiting body is convex, more or less circular, smooth, dark brown and fleshy, whitish on the under surface. It measures 3 to 10 cms. in diameter. It is sessile and attached to the substratum by rhizoids or tufts of hyphae. The asci are cylindrical and contain eight hyaline fusiform spores, measuring 9 to 10  $\mu$  by 32 to 36  $\mu$ . Numerous septate paraphyses are present among the asci (Masse<sup>67</sup>).

## Order: BASIDIOMYCETALES.

Most of the wood-destroying fungi belong to this order. The account given below comprises those families and species which have been shown to destroy timber and wood-pulp.

## Sub-order: EUBASIDIINEAE. A. HYMENOMYCETES.

Family: *Thelephoraceae*.*Coniophora cerebella*, Albertini et Schweinitz.Syn.: *Corticium puteaneum*, Schumacher.*Thelephora puteanea*, Schumacher.*Hypochnus confluentis*, Bonorden.*Corticium puteancum*, Fries.

This species has been shown by several investigators to be a serious destroyer of wood. Malenkovic<sup>68</sup> studied its action on wood in pure culture and found that it decomposed both cellulose and lignin, the latter more slowly than the former. Wehmer<sup>69</sup> confirms that it destroys cellulose, but states that it is incapable of utilizing lignin. Falck<sup>70</sup> regards *Coniophora*



*cerebella* as a particularly dangerous wood destroyer, since its mycelium develops exceptionally rapidly. Badly seasoned wood appears to be particularly susceptible to attack. The optimum moisture conditions for the fungus are between 50 and 60 per cent. (Scheible<sup>71</sup>), and is thus somewhat higher than that of most wood-destroying forms. Scheible also mentions that wood which has been invaded by *Coniophora cerebella* is particularly liable to attack by the dry rot fungus, *Merulius lacrymans*.

The mycelium of *Coniophora cerebella* is white when young, later turning yellowish-grey to brown. It contains a considerable amount of resin. Numerous clamp connexions, sometimes five or more, are formed at the septa. Granular deposits, possibly of calcium oxalate, are formed on the outer walls of the hyphae.

The sporophore is rounded or spreading, fleshy and brittle, faintly yellowish when young, and later becoming olive-brown. The hymenium is slightly undulating and is covered with a dusty layer of brownish-olive spores measuring 8 to 9  $\mu$  by 12 to 16  $\mu$ .

In addition to basidiospores the fungus produces oidiospores.

*Coniophora tabacina*, Sowerby.

Syn. : *Stereum tabacinum*, Sowerby.

*Auricularia tabacina*, Sowerby.

*Auricularia nicotiana*, Bolton.

*Thelephora variegata*, Schrader.

*Thelephora ferruginea*, Persoon.

*Thelephora tabacina*, Fries.

*Stereum tabacinum*, Fries.

The action of a pure culture of this fungus on wood was studied by Bray and Andrews<sup>72</sup>, who found that it had a selective action on cellulose, the lignin of the wood remaining practically undamaged (see Chapter XII).

The sporophore is soft, spreading, leathery, thin, and resupinate. When young it is covered with delicate hairs which disappear with age. The margin is golden yellow, and the central part reddish-brown. The hymenium is pale and covered with stiff hairs. The hyaline egg-shaped spores measure 1  $\mu$  by 3 to 5  $\mu$ .

*Corticium evolvens*, Fries.

Syn. : *Thelephora evolvens*, Fries.

This is claimed by Lagerberg<sup>73</sup> to be one of the most destructive fungi met with on wood used for wood-pulp manufacture.

The sporophore is soft, the edge often reflexed, and the under surface covered with a whitish undifferentiated fleecy layer. The hymenium is naked, somewhat wrinkled and brown, faded when old. When dry it shows numerous cracks.

*Peniophora gigantea*, Massee.

Syn. : *Corticium giganteum*, Fries.

*Thelephora gigantea*, Fries.

*Thelephora pergamena*, Persoon.

*Thelephora fimbriata*, Sommerfelt.

Was reported by Snell<sup>74</sup> to be present on hard pine beams in cotton warehouses. Nothing is known as to the extent of the damage caused by it.

The sporophore is moist, swollen, and spreading, waxy, white, and transparent. When dry it is of the consistency of cartilage or parchmented paper. Its periphery is frayed. The hymenium is smooth and undifferentiated. The hyaline spores are elliptical, measuring  $3\ \mu$  by  $4$  to  $5\ \mu$ .

*Peniophora pubera*, Saccardo.

Syn. : *Corticium puberum*, Fries.

*Thelephora pubera*, Fries.

*Hyphoderma pubera*, Wallroth.

This species is recorded by Snell<sup>74</sup> as occurring on badly decayed wood, generally when the latter is exceptionally moist.

The sporophores are spreading, waxy, white to buff in colour, and adhere firmly to the substratum. The hymenium is smooth and velvety, and extensively cracked when dry.

*Stereum frustulosum*, Fries.

Syn. : *Thelephora frustulata*, Persoon.

*Thelephora sinuans*, Persoon.

Von Schrenk and Spaulding<sup>75</sup> consider this species to be one of the chief oak-destroying fungi, responsible for the loss of large quantities of structural timber. Its action is not confined to oak, however, other broad-leaf trees, both living and dead, also being attacked. It attacks lignin for preference, and leaves the cellulose apparently undamaged. It develops in patches on the attacked wood, which becomes flecked with white lens-shaped pockets of decay. Such wood is known as

'partridge wood'. These white patches are the regions in which the fungus has removed the lignin of the wood. In older samples of attacked wood the white patches collapse and form pits lined with white cellulose. The fact that the attacked areas collapse in this way doubtless indicates that the cellulose, as well as the lignin, has been affected by the fungus. The unattacked parts of the wood become darker and very hard.

The sporophores are slightly elevated grey spots, woody and massed together to resemble one much-cracked specimen. The under surface and the glabrous margin are brownish-black in colour. The substance of the fungus is distinctly stratified. The hymenium is convex, and cinnamon in colour, later turning to pale primrose. The spores are elliptical, with sub-acute ends, and measure  $4.5 \mu$  by  $3$  to  $3.5 \mu$  (Massee<sup>67</sup>).

*Stereum hirsutum*, Willdenow.

Syn. : *Thelephora hirsuta*, Willdenow.

*Auricularia reflexa*, Bulliard.

*Thelephora papyracea*, Flora danica.

*Stereum hirsutum*, Persoon.

*Auricularia aurantiaca*, Schumacher.

A common saprophyte, which does, however, occur in some localities as a parasite. Its usual host is oak, either in the form of logs or of worked timber. It may also occur on beech (Tuzson<sup>63</sup>) and on the woods used for aeroplane manufacture (Boyce<sup>76</sup>). The fungus decomposes lignin and therefore produces what is known as a white rot. The attacked wood progressively becomes pale brown and yellowish-white in longitudinal streaks.

The sporophores are hard and leathery, expanding and resupinate. They are covered with rough hairs in zones. The blunt margin is yellowish, the remainder paler. The hymenium is smooth, naked, dry, and usually yellowish in colour. The spores are spherical and very small.

*Stereum purpureum*, Persoon.

Syn. : *Auricularia persistens*, Sowerby.

*Thelephora purpurea*, Schumacher.

This species may live as a saprophyte or as a wound parasite. It occurs on such trees as elm, beech, poplar, and sometimes

larch, but not on oak. Tuzson<sup>63</sup> records that it produces a white rot in freshly felled beech.\*

The sporophores, which show an imbricated structure, are soft, leathery, spreading, and resupinate, felty and pale or whitish. The hymenium is naked, smooth, and purplish in colour. The hyaline spores are oval to egg-shaped, pointed at the base, and measure 4 by 8  $\mu$ .

*Stereum sanguinolentum*, Albertini et Schweinitz.

Syn.: *Thelephora sanguinolenta*, Albertini et Schweinitz.

*Thelephora hirsuta*  $\beta$ , Persoon.

*Thelephora sericea*  $\beta$ , Persoon.

*Stereum sanguinolentum*, Fries.

The action of this species on timber was studied by Lagerberg<sup>73</sup>, who found it to be one of the most destructive fungi in wood stored for pulp manufacture. It is the rapid spread of the infection which makes this fungus particularly dangerous: the actual destruction of the timber proceeds comparatively slowly.

The sporophores are thin, leathery, spreading, and resupinate, and are covered with closely appressed silken hairs. The margin is sharp and white, the centre pale and somewhat striate. The hymenium is smooth, naked, and grey-brown, turning blood-red when touched. The hyaline spores are cylindrical and measure 3  $\mu$  by 8 to 10  $\mu$ .

*Stereum subpileatum*, Long.

This species, causing 'Honeycomb heart rot', occurs on felled trees and dead parts of living trees of the genus *Quercus*. The rot caused by this species has been studied in detail by Long<sup>77</sup>. The attacked wood first becomes slightly discoloured and assumes a 'water-soaked' appearance. Later it becomes tawny in colour and light-coloured cavities or pockets start to appear. The decay spreads most rapidly in the summer wood of the preceding year. Delignification of the wood proceeds until white lens-shaped pockets are formed. Long also remarks that the spread of a diseased area is arrested when it reaches a large medullary ray. This is interesting and exceptional, as the contents of the medullary ray cells are

\* Private information from Mr. F. T. Brooks indicates that delignification is very rarely caused by this species.

usually particularly susceptible to the attack of fungi. In the last stages of decay the wood becomes very light and honeycombed.

The hyphae of the fungus turn brown when exposed to air. The sporophores are thin shelving bodies formed in cracks in the bark. They are sometimes concoidal in form and sometimes occur in parallel lines, measuring up to 5 cms. in width (Harshberger<sup>78</sup>).

Family: *Polyporaceae*.

*Daedalea confragosa*, Bolton.

Syn.: *Boletus confragosus*, Bolton.

*Boletus labyrinthiformis*, Bulliard.

*Daedalea confragosa*, Persoon.

This species is considered by Snell<sup>79</sup> to be of economic importance in the decay of building timber. The presence of cellulase and hemicellulases in its mycelium was demonstrated by Schmitz and Zeller<sup>80</sup>.

The leathery to corky sporophore dries up in the absence of water and swells again and re-discharges its spores on the return of moist conditions.

The central part shows superficial zones of brown and cinnabar-red. The margin is white and frayed. The pores are closely set, labyrinthine, white, and later reddish with black spots.

The fungus forms chlamydospores, singly or in groups.

*Daedalea quercina*, Linnaeus.

Syn.: *Agaricus quercinus*, Linnaeus.

*Agaricus labyrinthiformis*, Bulliard.

*Agaricus dubius*, Schaeffer.

*Merulius quercinus*, Gmelin.

*Daedalea quercina*, Persoon.

This species is widely distributed on old oak trunks and stumps and is a serious enemy of structural oak. It attacks the sapwood, rendering it soft and mushy.

The sporophore is very irregular in size and shape. It is usually partly sessile, occasionally resupinate and spreading, somewhat wrinkled, without zones, smooth and of corky consistency, and buff-coloured. The dissepiments are thick with a blunted edge. They form holes when young and later anastomosing labyrinthine cavities.

*Fomes annosus*, Fries.

Syn.: *Polyporus annosus*, Fries.

*Polyporus subpileatus*, Weinmann.

*Polyporus serpentarius*, Persoon.

*Polyporus resinosus*, Rostkovius.

*Polyporus scoticus*, Klotzsch.

*Trametes radiciperda*, Hartig.

This fungus occurs on coniferous wood. It is widely distributed and causes a white pocket rot. In the early stages of attack the wood is discoloured pinkish, purple, or yellowish-brown. Where the rot has proceeded further the attacked wood becomes light and spongy. Von Schrenk<sup>81</sup> states that the decayed wood smells strongly of hydrocyanic acid.

The fructification is irregular in form, often horizontal and imbricated, measuring 7 to 15 cms. across. The pileus is convex, later becoming flattened. It is tuberculately zoned, coarsely radially rugulose, brown in colour, thickish, and has a white margin. The flesh is whitish, the tubes white, about 6 mms. long and stratoose, the pores are white, and the spores, measuring 6 by 4  $\mu$ , are hyaline (Massee<sup>67</sup>).

*Fomes applanatus*, Persoon.

Syn.: *Boletus applanatus*, Persoon.

*Polyporus applanatus*, Wallroth.

*Polyporus dryadeus*, Rostkovius.

*Polyporus merismoides*, Corda.

Is regarded by von Schrenk and Spaulding<sup>76</sup> as a strict saprophyte. They state that where it occurs on standing trees only the dead parts are attacked.\* It affects both heartwood and sapwood, with a preference for the latter (von Schrenk and Spaulding). Both coniferous and broad-leaf trees are attacked. The decay caused is described as a white mottled rot.

The sporophores are hemispherical in form, with flattened sides, and reach a size of 33 cms. Their surface is faintly zonate, dusty or smooth and cinnamon-brown in colour, later turning grey. The surface layer is brittle, the interior soft and spongy. The margin is white in the younger stages and later becomes cinnamon-brown. The pores are very small, and a faded rust colour, and whitish at the openings, where they turn brown when touched.

\* Private information from Mr. F. T. Brooks indicates that the fungus often occurs as a wound parasite.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>82</sup>:

The mats of mycelium are skin-like with a felty to farinaceous surface, of various colours from pale ochre to greyish-olive. The spread is rapid, slopes becoming covered in twelve to fourteen days.

*Submerged mycelium*: The hyphae are delicate, hyaline, colourless, thin-walled, branched, and septate. They measure 4 to 10  $\mu$  in width, and show inconspicuous clamp connexions and large swollen cells often massed into compact layers at various depths. *Aerial mycelium*: (1) The advancing zone and part of the older mat is similar to the submerged mycelium, but without spherical expansions. (2) Fibre-like threads with thick walls and narrow lumina, 1 to 4  $\mu$  wide. They are branched, sparingly septate, tightly interwoven, and produce 'witches' brooms' in old cultures.

*Fomes fomentarius*, Linnaeus.

Syn.: *Boletus fomentarius*, Linnaeus.

*Polyporus fomentarius*, Fries.

This species, known as the 'tinder fungus', occurs saprophytically and parasitically, chiefly on beech, birch, maple, and poplar. Both heartwood and sapwood are attacked, with the formation of a white rot. The badly decayed wood is very soft and spongy, light yellow in colour and readily rubbed into a powdery mass of fibres.

The fructifications are hoof-shaped, measuring 10 to 20 cms. across and 10 to 15 cms. thick, with a greyish-brown upper surface which is concentrically grooved. The margin is greyish-white, soft and velvety when young. The flesh is thick, rather soft, and brown in colour. The tubes are long, stratosed, and rust coloured, with white pores which later turn brown. The spores are brown and measure 6  $\mu$  by 3.5 to 4  $\mu$  (Masse<sup>87</sup>).

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>82</sup>:

The mats of mycelium form thick close felts with a smooth surface becoming leathery to crust-like with age. Their colour varies from pinkish-buff to brownish, and they spread moderately quickly, slopes being covered in about three weeks.

*Submerged mycelium*: The hyphae are delicate, colourless, hyaline, much branched, septate, and measure 2 to 7  $\mu$  wide. They show numerous clamp connexions. *Aerial mycelium*: (1) The advancing zone and part of the older growth is similar to the submerged mycelium. (2) Fibre-like hyphae of uniform width, with thick colourless or greenish walls, and colourless to dark brown contents. They are sparingly branched, septate, and measure 1 to 5  $\mu$  in width.

*Fomes laricis*, (Jacquin) Murrill.

Syn.: *Boletus officinalis*, Villars.

*Boletus laricis*, Jacquin.

*Boletus purgans*, Gmelin.

*Polyporus officinalis*, Fries.

This species, the 'chalky quinine fungus', causes a decay in the heartwood of various coniferous trees. It also occurs parasitically. Boyce<sup>76</sup> refers to it as one of the fungi causing decay in aeroplane timber. It produces a typical brown rot, that is, it destroys the cellulose of the wood and causes little, if any, damage to the lignin.

The sporophore is hoof-shaped, thick, corky to fleshy in consistency, soft when young and later becoming tough to dry, spongy, readily crumbling to powder when crushed. It shows yellow and brown zones and concentric grooves. It is glabrous, yellowish-white, and has a hard cracked outer layer. The pores are delicate, short and yellowish, turning brown with age.

*Fomes pinicola*, Swartz.

Syn.: *Boletus pinicola*, Swartz.

*Boletus fulvus et semiovatus*, Schaeffer.

*Boletus igniarius*, Flora danica.

*Boletus marginatus*, Persoon.

*Polyporus pinicola*, Fries.

*Trametes pini*, Fuckel.

This species occurs occasionally on living trees (Fritz<sup>82</sup>), but is widespread as a saprophyte, particularly on coniferous timber, of which it attacks both heart- and sapwood. The decay produced is a brown rot or 'red rot'. In the advanced stages of decay the tissues of the attacked wood are completely destroyed.

When young the sporophores are cushion-shaped, but later become hoof-shaped. The outer layer is corky to woody, glabrous, and yellow-brown in colour, later becoming blackish. The margin is cinnabar-red, hard, and pale on the under surface. The pores are small, short, and pale ochre in colour.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>82</sup>:

The mats of mycelium are delicate, easily torn, loose, dry and downy. They later become woven into a soft felt with a smooth woolly surface. They are coloured white, tinted a pale buff to



yellowish-pink. They spread rapidly, slopes being covered in twelve to fourteen days.

*Submerged mycelium*: The hyphae are delicate, colourless, hyaline, branched, and septate. They measure 2 to 6  $\mu$  in width, and show numerous clamp connexions. The walls are thin and the contents coarsely granular. *Aerial mycelium*: (1) Advancing zone and part of older mat is similar to the submerged mycelium, but is less vigorous in appearance, particularly the part of the older mat. The mycelium measures 1 to 2  $\mu$  and occasionally 4  $\mu$  in width. (2) Fibre-like hyphae are abundant and form young mats. They are hyaline, colourless, 1 to 4  $\mu$  wide, thick-walled, uniform, sparingly branched, and unseptate.

*Fomes roseus*, Albertini et Schweinitz.

Syn.: *Boletus roseus*, Albertini et Schweinitz.

*Polyporus roseus*, Fries.

This species chiefly attacks coniferous wood. From the statements of most authors the decay produced appears to spread through both sap- and heartwood. Faull<sup>83</sup>, however, finds no evidence of a heart rot in the cases examined by him. The species is evidently (Boyce<sup>76</sup> and Snell<sup>74</sup>) a type very destructive to timber, particularly when the wood is stored under conditions of poor ventilation and high humidity.

The sporophore is triangular, 5 to 12 cms. broad, and 1 to 3 cms. thick at the base, tapering to a sharp edge. In consistency it is corky to woody and hard. It is a rose-red colour both inside and outside, and on the surface has a greyish-black tinge in addition. The pores are small, cylindrical, and rose-coloured.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>82</sup>:

The mats of mycelium are light and fleecy, later becoming closely woven, with an even woolly surface. The colour is at first white, but later becomes brownish. It spreads moderately rapidly, slopes becoming covered in about three weeks.

*Submerged mycelium*: The hyphae are delicate, colourless, hyaline, septate, and branched. They are 1 to 3  $\mu$  wide and show numerous clamp connexions. In old mats the hyphae are often very irregular in outline and septation, and average 6  $\mu$  in width. A few are deeply pigmented. *Aerial mycelium*: (1) Advancing zone is similar to the submerged mycelium. (2) The older growth is also partly similar. Many of the hyphae become deeply pigmented and anastomose freely. (3) Fibre-like hyphae are not developed during the first ten days, but when formed they are colourless, hyaline, and 2 to 3  $\mu$  wide. They have thick, refractive walls and are sparingly branched and septate.

*Lenzites abietina*, Bulliard.Syn.: *Agaricus abietinus*, Bulliard.*Agaricus senescens*, Willdenow.*Agaricus asserculorum*, Schrader.*Daedalea abietina*, Fries.*Lenzites abietina*, Fries.

Falck<sup>70</sup> regards this and the following species as two of the most dangerous rots of stored timber.

The sporophore is spreading and resupinate, frequently expanding in one direction only, and reaching 4 cms. or more in length. It is leathery in consistency. When young it is brown and felted, and later becomes grey-black and glabrous. The dissepiments are irregular and simple. They are indented and fringed and a bluish-grey colour.

*Lenzites sepiaria*, Wulfen.Syn.: *Agaricus sepiarius*, Wulfen.*Agaricus betulinus*, Linnaeus.*Agaricus hirsutus*, Schaeffer.*Agaricus quercinus*, Humboldt.*Merulius squamosus*, Schrader.*Merulius sepiarius*, Schrank.*Daedalea sepiaria*, Swartz.*Agaricus Boletiformis*, Sowerby.*Lenzites sepiaria*, Fries.

Is a common and dangerous saprophyte, which occasionally occurs parasitically. It produces little or no external mycelium (Mitchell<sup>84</sup>). It gives rise to a brown cubical rot (Spaulding<sup>85</sup>), and therefore destroys the cellulose of the wood in preference to the lignin, which is only partly affected. Spaulding states that the species decomposes the coniferin and hadromal of the lignin, but does not affect the vanillin.

The sporophores are semi-sessile, fairly flat, and frequently grouped in masses. They are hard and leathery in consistency, with a zonate upper surface. They are brown in colour, later becoming blackish, and have a yellowish margin. The dissepiments are fairly thick, frequently indented, and are branched and anastomosing. They are yellowish in colour, later turning brown. Snell<sup>74</sup> states that there are morphological variations when the fungus grows on timber in sheds. The sporophores are occasionally, but not usually, pileate, and are normal in colour. The hymenium may be lamellate, poroid or daedaloid, and irpiciform if growing in wood that is too wet. The spores are usually smaller than

those from fructifications growing out of doors, measuring 6 to 9  $\mu$  by 2.5 to 3  $\mu$ , as against 8 to 12  $\mu$  by 2.5 to 4  $\mu$ .

The mycelium of the fungus shows clamp connexions and medallion hyphae, a common feature of the genus.

The following morphological characteristics of laboratory cultures on malt agar are given by Snell<sup>70</sup>:

The mycelium is colourless and consists chiefly of short-branched hyphae which break up more or less completely to oidia. Septa are fairly abundant, but no clamp connexions are observed. The oidia are colourless, sticky, mostly ellipsoid-oblong, but variable in form, and occasionally septate. Terminal oidia are usually clavate. They measure 2.5 to 3  $\mu$  by 4 to 35  $\mu$ . Helicoid hyphae are present but not abundant. When old the growth becomes brownish. The surface of the mycelium has a more or less damp-powdery appearance, due to the breaking up into oidia. Occasionally a later growth of mycelium occurs, which is long, stiff, and hair-like. This mycelium is sparingly branched, occasionally septate, and shows clamp connexions at the septa.

*Lenzites trabea*, Persoon.

Syn.: *Agaricus trabeus*, Persoon.

*Daedalea trabea*, Fries.

*Lenzites trabea*, Fries.

This species is usually found on hard woods, in which it causes a brown cubical rot. It is regarded by Snell<sup>70</sup> as a more dangerous infection of building timber than is generally assumed.

The sporophores are sessile, flat, wrinkled, very thin, and of leathery consistency. The surface is felted when young, but later becomes glabrous. The dissepiments are rigid, unbranched or dichotomously branched, occasionally anastomosing and flesh-coloured.

The following morphological characteristics of laboratory cultures on malt agar are given by Snell<sup>70</sup>:

The mycelium is colourless, 1.5 to 3  $\mu$  in diameter, and commonly branched, usually at right angles. Clamp connexions and septa are fairly abundant. Oidia are abundant, being mostly cylindrical to ellipsoid-oblong. The terminal oidia are ovoid, pyriform, clavate to globoid. The oidia measure 2 to 8  $\mu$  by 6 to 24  $\mu$ , but mostly 5 by 10  $\mu$ . Macroscopically the mycelium has a damp-powdery appearance, due to the formation of oidia. Later a secondary mycelium forms, which is orange-yellow to light buff when viewed in mass. This mycelium does not present a powdery appearance, because no oidia are present; it is thick, fluffy and woolly. Microscopically the individual hyphae appear colourless,

and are long, straight, and stiff. Branching and septa are scarce, but clamp connexions are fairly abundant.

*Merulius lacrymans*, Wulfen.

Syn.: *Boletus lacrymans*, Wulfen.

*Merulius lacrymans*, Schumacher.

*Boletus arboreus*, Sowerby.

*Merulius destruens*, Persoon.

*Xylomyzon destruens*, Persoon.

This species is the well-known 'dry rot' which causes serious damage to structural timber in badly ventilated places. It is a typical saprophyte, producing a brown cubical rot in all kinds of wood. In addition to the cellulose, it decomposes the hemicelluloses (xylan) of the attacked wood (Schorstein<sup>86</sup>). The lignin is probably not acted upon, at least not when of the constitution tested by Wehmer<sup>69</sup>. The oxidation of the cellulose and other carbohydrates of the wood results in the formation of oxalic acid, which is extensively deposited as calcium oxalate on the outside of the hyphae and the hyphae-bundles.

The optimum moisture content of wood for the growth of the fungus is given as 20 per cent. by Scheible<sup>71</sup>, who also remarks that a preliminary attack by *Coniophora cerebella* renders wood particularly liable to destruction by *Merulius lacrymans*. On the other hand the presence of bacteria and fungi was found to be unfavourable for the development of *Merulius lacrymans* by Malenkovic<sup>87</sup> who considered that the good development of the spores of the fungus on the acid-reacting media tried was due to the absence of bacteria.

The optimum temperature for growth is 22° C., development ceasing at 27° C. (Falck<sup>70</sup>). In this respect *Merulius lacrymans* differs from most wood-destroying fungi, including the closely related *Merulius silvestris*, which continues to develop at temperatures up to 34° C. The mycelium of *Merulius lacrymans* is killed at 38° C.

During growth the mycelium exudes drops of liquid, probably containing soluble decomposition products of the attacked wood. It is the exudation of these droplets which has given rise to the specific name of the fungus.

The fungus is able to spread over great distances, even

where insufficient moisture is available to support growth. This is due to the development of bundles of hyphae, 'rhizomorphs', which act as water-conducting organs which supply the mycelium growing in dry places with the necessary moisture. Falck<sup>70</sup> contends that the nature of the rhizomorphs is not that of a water-supply system, and states that *Merulius lacrymans* produces sufficient moisture by the decomposition of the wood to satisfy its water requirements and even to render the surrounding atmosphere damp. This statement undoubtedly requires confirmation. The rhizomorphs are described in somewhat greater detail in Chapter XII.

According to the functions which the hyphae of the fungus have to fulfil, they are differentiated into three structural types. These are the ordinary thin-walled hyphae, the water-conducting hyphae mentioned above, and a sclerenchymatous type possessing very thick walls. Clamp connexions are present in the hyphae and chlamydospores are formed.

The fructification of *Merulius lacrymans* consists of a thick felt-like crust, 10 to 20 cms. across, attached throughout to the substratum. It sometimes assumes a bracket-like and imbricate appearance. The surface is brownish in colour, and covered with low anastomosing wrinkles, over which the hymenial layer is spread. Four short sterigmata on each basidium bear the spores, which are deep brown or snuff-coloured, and give the hymenium a powdery appearance. The spores, which measure 9 to 12  $\mu$  by 5.5 to 6.5  $\mu$ , are dish-shaped, with the convex side turned outwards while they are on the sterigmata.

The whole fructification is surrounded by a mass of white mycelium resembling cotton-wool and later turning dull grey. Sometimes the entire hymenium remains sterile, in which case it does not turn brown but remains a dirty grey colour. (Description based on Harshberger<sup>78</sup>, Massee<sup>67</sup>, *et alii*.)

*Polyporus adustus*, Willdenow.

Syn.: *Boletus adustus*, Willdenow.

*Boletus suberosus*, Batsch.

*Boletus pelleporus*, Bulliard.

*Boletus concentricus*, Schumacher.

*Poria argentia*, Ehrenberg.

*Polyporus adustus*, Fries.

This species occurs commonly in dead sapwood of the red gum tree (*Liquidamba styraciflua*) and on the dead sapwood of other broad-leaf trees (von Schrenk<sup>88</sup> and von Schrenk

and Spaulding<sup>75</sup>). It is responsible for a white spongy rot of the attacked wood.

The imbricated sporophores are irregular in shape and size, and broader at the base. They are thin, but tough and fleshy in consistency and faded grey in colour. The margin is stiff and blackish. The pores are short, small, and rounded. They are whitish when young, but later become greyish-brown. The mycelium of this fungus shows clamp connexions.

*Polyporus amorphus*, Fries.

Syn.: *Polyporus nitidus*, Albertini et Schweinitz.

*Boletus irregularis*, Sowerby.

*Boletus abietinus*, A. P. de Candolle.

*Polyporus aureolus*, Persoon.

*Polyporus rosco-poris*, Rostkovius.

This species and *Polystictus abietinus* do a great deal of damage to pitch pine in Pennsylvania, causing a rot which eliminates most of the cellulose, and in the later stages also some of the lignin (Overholts<sup>89</sup>).

The sporophores are spreading, resupinate, and very irregular in shape. They are often imbricated, or merging one into another. They are thin, tough, fleshy, white in colour, and covered by delicate hairs. The pores are small, irregular, and golden yellow to reddish in colour.

*Polyporus balsameus*, Peck.

This occurs on fallen trunks of coniferous trees, in which it produces a brown cubical rot of the heartwood.

The pileus is rather thin, corky, plain, 2 to 3 cms. broad, sessile or sometimes possessing a false stipe, downy and pale brown with lighter concentric zones. The flesh is white and the pores are short, minute, and subrotund. The dissepiments are thin, acute, denticulate, and white (Peck<sup>90</sup>).

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>92</sup>:

The mats of mycelium are downy, with a broad, colourless, appressed, advancing zone. Later they become more woolly and finally assume a farinaceous texture. The colour is white gradually becoming drab, with occasional honey-coloured patches. The spread is moderately rapid, slopes becoming covered in the third week.

*Submerged mycelium*: (1) The hyphae are delicate, colourless, and 2 to 6  $\mu$  wide, with firm hyaline walls. They are branched and septate, and in old cultures produce large conspicuous and

irregular expansions which occur both singly and in series. Numerous clamp connexions are present: (2) Chlamydospores are developed during the first week, usually in the fine hyphae about  $2\mu$  wide. They are elliptical to subspherical, measuring 10 by  $12\mu$ , or may be irregular, ranging from 7 to  $10\mu$  by 12 to  $22\mu$ . They may be terminal or intercalary, and have thick, smooth, hyaline walls and colourless to yellowish contents. *Aerial mycelium*: (1) The advancing zone and part of the older mat are similar to the submerged mycelium. (2) Chlamydospores are often produced terminally, in spray-like clusters, but also in intercalary positions, forming chains. Their contents are yellowish to deep brown. (3) Coarse hyphae are produced which are similar in type to the above, but have thicker walls and frequent sac-like expansions which are filled with dense, drab-brown contents.

*Polyporus borealis*, Wahlenberg.

Syn.: *Boletus borealis*, Wahlenberg.

*Boletus albus*, Schaeffer.

*Polyporus borealis*, Fries.

This species is regarded by Haas,<sup>91</sup> as an important type responsible for the decay of coniferous wood and pulp.

The sporophores, which last for only one season, are bracket-like, soft, and spongy, and are usually imbricated. They measure 10 to 20 cms. by 6 to 15 cms. broad, and are attached by a broad base, or narrow into a short, more or less distinct, stem. The upper surface is whitish, rough, often more or less radially wrinkled, somewhat hairy, and sodden in appearance. The pores, on the underside, may be regular with rounded openings, or irregular, elongated, and wavy, with the dissepiments torn. The spores are colourless and subglobose. They measure  $4\mu$  in diameter. (Description based on Harshberger<sup>78</sup> and Massee<sup>97</sup>.)

Hubert<sup>92</sup> states that clamp connexions are absent, but Fritz<sup>92</sup> finds them present in laboratory cultures.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>92</sup>:

The mats of mycelium are thick and soft, and have a felty, velvety surface. Felty balls and ridges are formed over the surface. Sporophores are not infrequently formed. The mats are white or creamish in colour, and spread slowly, slopes being covered in five weeks. The mats are thick, however.

*Submerged mycelium*: (1) The hyphae are fine, regular, 2 to  $6\mu$  wide, and much branched. They are septate, show abundant clamp connexions, and have firm, colourless, hyaline, and refractive walls. (2) Chlamydospores are abundant, and vary in form from spherical to pear-shaped or irregular. They have smooth hyaline walls, coarsely granular contents, and are 6 to  $10\mu$  by 10 to  $20\mu$  in size. *Aerial mycelium*: (1) The advancing

zone and part of the older mat are similar to the submerged mycelium. (2) Chlamydospores as above. (3) Fibre-like hyphae develop in old cultures and have thick walls and narrow lumina. They are sparingly branched and septate, often irregular in outline, and show infrequent clamp connexions.

*Polyporus destructor*, Schrader.

Syn.: *Boletus destructor*, Schrader.

This species occurs chiefly on structural coniferous wood in damp places. The rot for which the fungus is responsible causes a softening of the wood not unlike that produced by 'dry rot'.

The fructifications consist of patches 7 to 15 cms. long which are effused or partly reflexed. They are fragile, rugose, brownish-white in colour, rather fleshy and 'watery-zoned'. The tubes are about 1 cm. long, the pores white and rounded. The dissepiments become torn into teeth at the margin. The tubes form nearly the whole of the fruit body (Massee<sup>67</sup>).

*Polyporus ponderosus*, von Schrenk.

Has been described by von Schrenk<sup>93</sup> as the cause of a brown rot of *Pinus ponderosa*, known in America as the western yellow pine. Haas<sup>91</sup> regards the fungus as a dangerous type, decaying wood stored under rather warm and moist conditions.

The sporophores start as flesh-coloured knobs which increase in size and turn reddish, forming brackets. These occur singly or in groups of two or three, and may continue to grow for some years, a ring being added on the outside when new growth commences. The upper surface is rough, and appears as if covered with a waxy substance which has hardened and cracked. The lower surface is smooth with regular pores (Harshberger<sup>78</sup>).

*Polyporus squamosus*, Hudson.

Syn.: *Boletus squamosus*, Hudson.

*Boletus caudicinus* var. 1, Scopoli.

*Boletus cellulosus*, Lightfoot.

*Boletus juglandis*, Bulliard.

*Boletus platyporus*, Persoon.

*Polyporus squamosus*, Fries.

*Polyporus giganteus*, Harzer.

*Polyporus flabelliformis*, Persoon.

Is responsible for a white rot of most dicotyledonous trees except the oak (Buller<sup>94, 95</sup>).



The sporophores are usually imbricated, or several arise from one point on a more or less lateral stem. They are fan-shaped or nearly circular, fleshy behind, and becoming thin towards the margin. They are usually 10 to 30 cms. across, though specimens up to 65 cms. across have been recorded. Growth is very rapid. The substance of the fruit body is at first soft and juicy, later drier and very tough. The upper surface is dingy yellowish-white, with darker appressed scales, giving a roughened appearance. The pores are large, irregular in form, and whitish, with a short stem and blackish base, the pores running down the stem (Masse<sup>67</sup>). The spores are egg-shaped, hyaline, and 5 by 12  $\mu$  (Rabenhorst<sup>5</sup>). The hyphae of this fungus show clamp connexions.

*Polyporus subaculus*, Peck.

Syn.: *Porus subacida*, Peck.

Produces a white rot which is common on the fallen trunks of coniferous and other trees, and on felled timber (von Schrenk).

The mycelium grows out over the bark and forms yellow felts. A few weeks later small pores begin to form. Certain hyphae of the sheet turn out at right angles and grow out to form shallow pores, which are almost round and separated by thin dissepiments. No pores are formed at the edge of the sheet, a fringe of sterile hyphae being left. This distinguishes the species from many allied forms. The hymenial layer and the pores are straw yellow (von Schrenk).

The pores are minute and often oblique.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>82</sup>:

The mats of mycelium are at first light, flocculent-downy, with a broad appressed advancing zone. They later become woolly. Their colour is at first white, but later yellowish. The spread is rapid, slopes becoming covered in two weeks.

*Submerged mycelium*: The hyphae are delicate, colourless, and 2 to 6  $\mu$  wide, with firm hyaline walls and coarse granular contents. Clamp connexions are numerous. *Aerial mycelium*: (1) The advancing zone consists of vigorous turgid hyphae similar to the submerged. (2) Part of the older growth is similar to the submerged. (3) Fine fibre-like hyphae, which are 1 to 3  $\mu$  wide and sparingly branched and septate. They have thick hyaline walls and narrow lumina, at first having granular contents, but soon becoming empty.

*Polyporus sulphureus*, Bulliard.

Syn.: *Boletus sulphureus*, Bulliard.

*Boletus caudicinus* var. 2, Scopoli.

*Boletus coriaceus*, Hudson.

*Boletus tenax*, Bolton.

*Boletus lingua cervina*, Schranck.

*Boletus citrinus*, Planer.

*Sistotrema sulphureum*, Rebentisch.

*Polyporus sulphureus*, Fries.

*Polyporus Todari*, Inzenga.

Although frequently a parasite, this fungus may attack the heartwood of dead trees (Boyce <sup>76</sup>), and also structural timber (von Schrenk and Spaulding <sup>75</sup>). It is responsible for a brown cubical rot.

The sporophores occur in groups, either tufted or imbricated, and form masses 20 to 100 cms. across. The fruiting bodies are usually sessile, but if developing on the underside of a log they may have distinct stipes. The individual parts are comparatively thin wavy plates. The upper surface is sometimes rather hairy, very moist, and yellow or orange in colour, being brighter at the margin, and turning brown when bruised. The flesh is soft, yellow, and later whitish, is of a cheese-like consistency, and contains a clear yellow liquid when young. The lower surface is sulphur yellow, soft, and even. The pores are minute and the spores elliptical and minutely warted. They measure 7 to 8  $\mu$  by 4 to 5  $\mu$  (Masse <sup>67</sup>).

Numerous drops of liquid sometimes exude from the under surface of the shelves; this liquid contains melezitose, a sugar produced by the fungus.

Hubert <sup>82</sup> noted the presence of clamp connexions in the hyphae of the fungus, but Fritz <sup>82</sup> does not record their presence in laboratory cultures.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz <sup>82</sup>:

The mats of mycelium are soft and downy, becoming spongy with age, and are coloured white at first and later turn to ochraceous salmon. The spread is moderately fast, slopes becoming covered during the third week of growth.

*Submerged mycelium*: (1) The hyphae are rather delicate, hyaline, loosely branched, frequently septate, and often constricted at the septa, but showing no clamp connexions. They vary in width from 3 to 12  $\mu$  and, in old cultures, up to 22  $\mu$ . (2) The chlamydospores are irregular in outline, terminal or intercalary, 10 to 13  $\mu$  by 18 to 30  $\mu$  in size, and have relatively thin walls. *Aerial mycelium*: (1) The advancing zone and most of the older growth of hyphae are similar to those in the submerged layers. (2) The chlamydospores are borne terminally, in spray-like clusters, or are formed in series in intercalary positions. In the former case they are subspherical and in the latter varied in outline. They measure

6 to 7  $\mu$  by 6 to 11  $\mu$  in size. (3) Fine fibre-like hyphae are found in old cultures. They are 1 to 3  $\mu$  wide, have thick walls and narrow lumina, and show infrequent branching and septation.

*Polyporus vaillantii*, A. P. de Candolle.

Syn. : *Boletus vaillantii*, A. P. de Candolle.

*Polyporus vaillantii*, Fries.

This fungus was found by Mitchell<sup>84</sup> to be a common cause of rot in coal-mine timber.

The fructification is thin, white, and spreading. The mycelium is entangled to form a ribbed membrane on which clusters of short, fairly large, delicate, and irregular pores arise.

*Polyporus vaporarius*, Persoon.

Syn. : *Poria vaporaria*, Persoon.

*Boletus vaporarius*, Persoon.

*Polyporus vaporarius*, Fries.

*Polyporus incertus*, Persoon.

Is a very common saprophyte on all types of wood. The rot caused by this fungus, a cubical brown rot of the sap-wood, greatly resembles that produced by *Merulius lacrymans* and has probably been frequently mistaken for it. The fructifications, however, when present, are sufficiently diverse in appearance to make the differentiation possible. Haas<sup>91</sup> considers that *Polyporus vaporarius* is particularly dangerous where wood is stored under rather warm conditions.

The sporophores are spreading and crust-like, and adhere to the substratum. The mycelium is tufted, white, and on larger surfaces often forms anastomosing strands. The pores are fairly large, angular and white, forming a tough, lasting layer. The spores are longish, colourless, and slightly curved.

The fructifications tend to be abnormal when occurring in buildings.

*Polyporus volvatus*, Peck.

The rot produced by this fungus is described by Meinecke<sup>96</sup> as a slowly progressing and rather superficial grey rot. This probably implies that the lignin of the attacked wood becomes largely destroyed. That the cellulose present is also attacked is clear from the observations of Schmitz<sup>97</sup>, who found that both cellulase and hemicellulases are produced by this species.

The annual fruiting bodies are rather small, light yellowish-brown when young, white with age, hoof-shaped, and corky to hard. The entire surface is very smooth. The underside, with its small pores, is hidden by a thick leathery skin which forms a pouch. The spores, which are pink in colour, escape through a small hole in this skin (Meinecke<sup>96</sup>).

*Polystictus abietinus*, Dickson.

Syn.: *Boletus abietinus*, Dickson.

*Boletus purpurascens*, Persoon.

*Boletus incarnatus*, Schumacher.

*Polyporus abietinus*, Fries.

*et alia*.

Is regarded by Haas<sup>91</sup> as an important type responsible for the decay of coniferous wood and pulp. Overholts<sup>89</sup> found it causing a brown rot on dead pitch pine in Pennsylvania (see also *Polyporus amorphus*, Fries).

The sporophores are spreading, resupinate, and often imbricated. They are leathery, thin, greyish-white in colour, and indistinctly zonate on the upper surface. The margin is frequently corrugated. The pores are angular and purplish when young, later becoming irregular, faded purple in colour, and merging into one another.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>82</sup>:

The mats of mycelium are at first thin, with a farinaceous surface. Later they become skin-like, with a felty-woolly surface and deep radiating furrows. Their colour is white, becoming sodden, and the spread is moderately rapid, slopes being covered during the third week.

*Submerged mycelium*: (1) The hyphae are hyaline, colourless, branched, septate, and 2 to 4  $\mu$  in width. They have firm walls and abundant conspicuous clamp connexions. *Aerial mycelium*: (1) The advancing zone is similar to the submerged mycelium. (2) The older growth is similar to the submerged, but with thicker walls, and shows a tendency to form short upright branches with many irregular enlargements. The hyphae are 3 to 5  $\mu$  wide.

*Polystictus pergamenus*, Fries.

Syn.: *Polyporus pergamenus*, Fries.

This species often occurs on standing trees which have been killed by forest fires. It will develop in the sapwood of practically all dead deciduous trees, causing a white rot.

The sporophores usually grow together, one over another, and joined laterally to form long series of shelves. The body of the

sporophore is leathery and rigid. The top is concentrically sulcate, usually white when young and greyish when older. The upper surface is slightly hairy. The lower surface is generally purplish in colour. The pores are small and the dissepiments become much torn and lacerated, so that in older specimens they resemble teeth or spines (von Schrenk and Spaulding <sup>75</sup>).

The mycelium of this fungus shows clamp connexions.

*Polystictus stipticus*, Persoon.

Syn.: *Polyporus stipticus*, Persoon.

*Boletus stipticus*, Persoon.

*Polystictus stipticus*, Fries.

This fungus is responsible for a white pocket rot. It was reported present in decayed structural timber of coal-mines by Mitchell <sup>84</sup>.

The fructifications are cushion-shaped and merge into one another to give an imbricated structure. They are about 12 cms. in width and 2 cms. thick, with a hook-like stem. They are of fleshy to corky and somewhat brittle consistency, with a smooth and white surface. The margin is blunt and reddish, and the pores are long, cylindrical, white in colour, and even in size.

*Polystictus versicolor*, Fries.

Syn.: *Boletus versicolor*, Linnaeus.

*Boletus atro-rufus et variegatus*, Schaeffer.

*Boletus imbricatus*, Scopoli.

*Boletus plicatus*, Schumacher.

*Polyporus versicolor*, Fries.

*Polyporus argyraceus*, Persoon.

*Polyporus stereoides et radiatus*, Rostkovius.

*Polyporus zonatus*, Rostkovius.

*Polyporus nigricans*, Lasch.

This is one of the most destructive fungi on dicotyledonous woods (Fritz <sup>82</sup>). Sooner or later all timber in contact with soil appears to become attacked by this fungus (von Schrenk and Spaulding <sup>75</sup>). Though the rot caused by it is described by Hubert <sup>82</sup>, Haas <sup>81</sup>, and Tuzson <sup>83</sup> as a white spongy rot, von Schrenk and Spaulding state that it decomposes both lignin and cellulose. Smith <sup>88</sup>, however, reports that it attacks all the carbohydrate constituents of the apple tree, but leaves the lignin undamaged.

The varicoloured sporophores occur singly or in dense masses forming series of overlapping shelves. They are very variable,

according to the conditions and the wood attacked. They are sessile, with a soft hairy upper surface resembling *Stereum hirsutum*, and showing alternate light and dark bands, usually white and yellow, but showing considerable variation in colour. The sporophores are fleshy when young, but tough and leathery later, when the front edge curls in also. The margin is irregular and wavy when dry. The lower surface is usually snow white and the pores are exceedingly regular and minute. The body of the sporophore is very thin, not exceeding the thickness of heavy paper (von Schrenk and Spaulding<sup>75</sup>).

Oidia and chlamydospores are formed by the mycelium. Hubert<sup>82</sup> found no evidence of clamp connexions in the mycelium in wood cultures, but Fritz<sup>82</sup> reports their presence in cultures on artificial media.

The following morphological characteristics of laboratory cultures on potato-dextrose agar are taken from Fritz<sup>82</sup>:

The mats of mycelium are downy when young, later becoming felty and finally chalky. They are white and later yellow. The spread is rapid, slopes becoming covered in twelve days.

*Submerged mycelium*: The hyphae are colourless, hyaline, much branched, and septate, with numerous clamp connexions. The hyphae are 2 to 6  $\mu$  wide, and in old cultures are often very irregular in outline and greatly enlarged. *Aerial mycelium*: (1) The hyphae are similar to those of the submerged mycelium. (2) Colourless fibre-like hyphae, which are 2 to 5  $\mu$  wide, have thick hyaline walls and narrow lumina. They show infrequent branching and septation, very infrequent clamp connexions, and frequent terminal and intercalary expansions with very thick walls.

*Poria atrosporia*, Ames.

This may be found in coniferous wood. It causes a brown cubical rot and sometimes limits its attack to the spring wood, while in other cases it destroys only the summer wood.

The sporophore is resupinate, broadly effused and easily separable. The margin is sterile, and pale umbrinous in colour. The hymenophore is porose, very fragile, and is friable when dry. The pores are 1 to 5 mms. deep, the dissepiments thin, and the mouths irregular to subrotund, 1 to 5 to the millimetre. The trama is pale umbrinous, but the pores are deep, fulgineous, because of the abundance of dark spores. The spores are oval, dark brown, and measure 4 to 5.5  $\mu$  by 8 to 10  $\mu$  (Ames<sup>89</sup>).

*Poria incrassata*, Burt.

Syn.: *Merulius incrassatus*, Berkeley et Curtis.

This species produces a brown rot in coniferous wood resembling that of *Merulius lacrymans*. Humphrey<sup>100</sup> states

that it is a dangerous enemy of structural coniferous woods, and that it sometimes attacks oak also. The optimum temperature is stated by him to lie between 24 and 28° C., though the mycelium will grow at 13° C. At 36° C. its development is checked.

The mycelium is whitish when young, but turns yellowish-olive to brownish with age. It forms fan-shaped sheets. The fruiting body is flat and attached to the surface of the substratum. It varies in appearance, being orange to olive when young, but later turning to sepia and then brownish-black or even black. No orange coloration is present in fructifications formed in the dark. The fructification is 1 to 2 cms. thick, fleshy when fresh and drying to a fragile collapsed black mass. The spores are oval in shape, dusky olive to brown in colour, and measure 6.5 to 7  $\mu$  by 8 to 10  $\mu$ . The fungus is often found in the sterile condition. Rhizomorphs are sometimes formed. They are small and white when young, becoming brownish-black later. They are often flattened, and lie closely appressed to the decayed wood. (Description based on Humphrey<sup>100</sup>.)

*Trametes carnea*, (Nees) Corda.

Causes a brown cubical rot in coniferous heartwood. It is regarded by Snell<sup>74</sup> and others as probably identical with *Fomes roseus*, Albertini et Schweinitz, the former being an annual and the latter a perennial type of the same species.

A description of *Fomes roseus* is given on p. 124.

*Trametes cinnabarina*, Jacquin.

Syn.: *Boletus cinnabarinus*, Jacquin.

*Boletus coccineus*, Bulliard.

*Polyporus cinnabarinus*, Fries.

*Trametes cinnabarina*, Fries.

This species is considered by Haas<sup>91</sup> to be an important destroyer of wood and pulp. It causes a brown or 'red' rot, especially in material stored under rather warm conditions.

The sporophore is flat to convex and measures up to 12 cms. across. It is corky and delicately hairy when young, but later becomes glabrous. It is indefinitely wrinkled, zonate, and of an intense cinnabar-red colour. The pores are cylindrical, of uniform dimensions and of the same cinnabar-red colour.

*Trametes mollis*, Sommerfelt.

Syn.: *Daedalea mollis*, Sommerfelt.

*Polyporus cervinus*, Persoon.

*Trametes mollis*, Fries.

This is another of the species which Haas<sup>91</sup> considers responsible for the decay of wood and wood-pulp stored under warm conditions. It was identified by Mitchell<sup>84</sup> as occurring in coal-mine timber.

The sporophores are resupinate, sometimes round, and 2 to 5 cms. broad, sometimes elongated up to 30 cms. They are thin, almost skin-like, and light buff in colour, later becoming brown. The under surface is downy and has a brownish margin which is reflexed on ageing. The pores are very irregular, wide, wavy, frayed, and often angular.

*Trametes odorata*, Wulfen.

Syn. : *Boletus odoratus*, Wulfen.

*Boletus annulatus*, Schaeffer.

*Polyporus odoratus*, Fries.

*Trametes odorata*, Fries.

This species was observed by Mitchell<sup>84</sup> in the timber of coal-mines.

The fructification is cushion-like, 5 to 8 cms. broad and of corky consistency. During the first year it is brownish-yellow in colour, but later becomes blackish-brown, felted, and concentrically furrowed. The margin is cinnamon-brown. The pores are cylindrical or oval and are also cinnamon-brown.

*Trametes serialis*, Fries.

Syn. : *Polyporus serialis*, Fries.

*Boletus contiguus*  $\beta$ , Albertini et Schweinitz.

*Polyporus contiguus*, Persoon.

*Polyporus scalaris et frustulatus*, Persoon.

This produces a brown cubical rot in coniferous woods. Snell<sup>74</sup> states that it may possibly be identical with *Fomes officinalis*.

The sporophores, which occur in rows, are spreading, resupinate, of corky consistency, wrinkled, and bluish-grey in colour. The margin is blunt and the pores are small, white, and irregular, particularly in the crust-like part of the sporophore which adheres to the substratum. The individual sporophores have a width of 1 to 1.5 cms. ; in rows they may reach a width of 0.3 to 1 metre.

The following morphological characteristics of laboratory cultures on malt agar are given by Snell<sup>79</sup> :

The mycelium is colourless and 1.5 to 3.6  $\mu$  in diameter. The septa and clamp connexions are fairly abundant, but fewer than in



the fruiting mycelium. Branching is irregular, not abundant, not necessarily at right angles, and forking is common. The hyphae are straight for the most part and the contents homogeneous. Anastomosis is occasional, and chlamydospores are few. They are colourless, elliptical, fairly thick-walled, and measure 4.5 to 10  $\mu$  by 7 to 21  $\mu$ . The later fruiting mycelium is colourless, 4 to 6.6  $\mu$  in diameter, unequal in thickness, and in much of it the contents have disappeared. There are abundant protuberances, round or bluntly pointed. Branching is common, usually at the clamp connexions. Both septa and clamp connexions are abundant, and anastomosis is common. The chlamydospores are as above.

Family: *Agaricaceae*.

*Armillaria mellea*, (Vahl) Quélet.

Syn.: *Agaricus melleus*, Flora danica.

*Agaricus obscurus*, Schaeffer.

*Agaricus annularis*, Bulliard.

*Agaricus stipitis*, Sowerby.

*Agaricus mutabilis*, Flora Batava.

Though generally a parasite, this fungus is found as a destructive agent of old tree-stumps and of pit-props in coal-mines. It produces a white rot, but the wood does not appear to be seriously affected (von Schrenk <sup>81</sup>). Haas <sup>81</sup>, however, regards it as a dangerous type. It belongs to the group of wood destroyers which render the attacked wood phosphorescent (Molisch <sup>25</sup>).

The pileus is oval to convex and expanded, sometimes with a slight elevation. It is smooth or adorned with pointed dark brown or blackish scales, especially in the centre. It is honey-coloured to dull reddish-brown. The margin is even or somewhat striate when old. The gills are adnate, or decurrent, and whitish in colour, sometimes with reddish-brown spots. The stipe is elastic, spongy, sometimes hollow, smooth or scaly. It is generally whitish, but sometimes grey or yellow above the ring and reddish-brown below. The cap is 4 to 15 cms. broad, and the stem 5 to 15 cms. long and 1 to 2 cms. thick (Harshberger <sup>78</sup>).

The spores are white and elliptical and measure 9 by 6  $\mu$ .

*Lentinus lepideus*, Fries.

Syn.: *Agaricus lepideus*, Fries.

*Agaricus squamosus*, Schaeffer.

*Amanita crispa*, Lamarek.

*Agaricus tessellatus*  $\beta$ , Albertini et Schweinitz.

*Agaricus tigrinus*, Schumacher.

*Lentinus squamosus*, Schröter.

Causes a brown cubical rot in structural timber (Mitchell<sup>84</sup> and Snell<sup>74</sup>). It produces an odour of turpentine in the attacked wood. The changes caused by the fungus in ground wood were studied by Bray and Andrews<sup>72</sup>. Falck<sup>70</sup> regards this as one of the most dangerous species of wood-destroying *Agaricaceae*.

The pileus is irregularly shaped, convex when young, later flattened, 5 to 12 cms. broad. It is somewhat eccentric, tough, fleshy, compact, ochre coloured, and covered with darker scales. The stem is thick, and bears felt-like scales and rhizoids. The gills are wavy, broad, frayed, striped, and whitish in colour. The spores are almost spherical and measure 2 to 3  $\mu$  in diameter.

The following morphological characteristics of laboratory cultures on malt agar are given by Snell<sup>70</sup>:

The mycelium is light cottony or felty when young, and exhibits a tendency to form zones of aerial mycelium. It turns brown with age and produces an aromatic odour. *Microscopical appearance*: The colourless mycelium is 2 to 3  $\mu$  in width, shows frequent branching and septation, but few clamp connexions. The chlamydospores are colourless, usually terminal and ellipsoid, and measure 8 to 14  $\mu$  by 10 to 29  $\mu$ . They are commonly empty and show secondary walls, due to the contraction of the contents. The later mycelium is usually colourless, with occasional hazel-coloured hyphae and showing a slight colour in mass. They are long, stiff, hair-like, and measure 2.2 to 5.6  $\mu$  in diameter. The chlamydospores are similar to those described above and are thick-walled. Irregular hyphae are common. Snell<sup>74</sup> reports the development of abnormal fructifications on specimens found in building structures.

*Paxillus panuoides*, Fries.

Syn.: *Agaricus panuoides*, Fries.

*Merulius lamellosus*, Sowerby.

*Agaricus lamellirugus*, A. P. de Candolle.

*Gomphus pezizoides*, Persoon.

*Merulius crispus*, Turpin.

*Agaricus croceolamellatus*, Letellier.

*Cantharellus Dutrochetii*, Montagne.

*Paxillus acherontius*, Schröter.

This fungus occurs on stumps of coniferous trees, and is regarded by Falck<sup>70</sup> as a dangerous wood-destroyer. It was

also identified by Mahood and Cable<sup>101</sup> in a sample of infected mechanical wood-pulp. The decomposition had not proceeded very far, but it indicated a selective action on the cellulose of the pulp.

The pileus is fleshy, mussel-shaped or mug-shaped, and 2 to 11 cms. broad. It is fleshy when young, but glabrous later, sessile or resupinate, of very irregular shape and whitish to dirty yellow in colour. The gills are closely set, branching, anastomosing towards the base, wavy, and yellowish in colour. The spores are yellow, spherical to elliptical, and measure 3 to 4  $\mu$  by 4 to 6  $\mu$ .

*Schizophyllum commune*, Fries.

Syn.: *Agaricus alneus*, Linnaeus.

*Agaricus multifidus*, Batsch.

*Agaricus radiatus*, Swartz.

Produces a white rot in freshly felled beech wood (Tuzson<sup>63</sup>).

The pileus is very thin, fan-shaped, greyish-white in colour, often lobed, downy, and 2 to 5 cms. broad. The gills are pale brown with a tinge of purple, and have the split portion recurved. The spores are of a dingy colour and measure 4 to 6  $\mu$  by 2 to 3  $\mu$  (Massee<sup>67</sup>).

## FUNGI IMPERFECTI.

Order: SPHAEROPSIDALES.

Family: *Sphaerioidaceae*.

*Chaetomella horrida*, Oudemans.

Was isolated from humus by Oudemans and Koning<sup>10</sup>. Van Iterson, jr.<sup>40</sup>, showed it to be a powerful cellulose decomposer, attacking this carbohydrate and forming delicate black hairy pycnidia on the cellulose discs used by him.

The growth consists of a creeping branched mycelium, at first white and later turning brown. The perithecia, which measure 140 by 180  $\mu$ , arise sparingly on the surface of the growth. They are ovoid, closed and brownish in colour. The perithecium possesses long erect hairs which are black and opaque at the base, and pale brown to pale olive above. They are smooth when young, but become rough on ageing, and show a system of

dichotomous branching. The elliptical spores taper towards the ends and measure  $5.5$  to  $7\ \mu$  by  $3.5$  to  $4\ \mu$  (Oudemans and Koning<sup>10</sup>).

*Pyrenochaeta humicola*, Oudemans.

This is stated by van Iterson, jr.<sup>49</sup>, to be a moderately active cellulose decomposer, rendering the attacked cellulose a dark colour. It is interesting to note that he found the fungus developed best at alkaline reactions.

The pycnidia are black,  $250\ \mu$  in diameter, and have an opening  $20$  to  $25\ \mu$  in diameter. The surrounding hairs are blackish-brown and  $330\ \mu$  in length. The spores are elliptical, hyaline, contain globules, and measure  $2.3$  to  $2.5\ \mu$  (Saccardo<sup>9</sup>).

Order: HYPHOMYCETALES.

Family: *Mucedinaceae*. Sub-family: *Hyalosporae*.

Subdivision: *Oosporae*.

*Monilia sitophila*, (Montagne) Saccardo.

This was studied by Gerry<sup>50</sup> and was shown by him to be capable of perforating the cell walls of woody tissues. The species is described under Group A (Chapter V).

Subdivision: *Cephalosporiæ*.

*Trichoderma Koningii*, Oudemans.

This type, which Oudemans and Koning<sup>10</sup> reported to be one of the most common humus inhabitants in the forest of Spanderswoude, was shown by Koning<sup>102</sup> to possess cellulose-digesting properties.

This species forms a circular woolly growth which is white at first, later becoming greenish to olive. The hyaline hyphae are sparingly septate with opposite or alternate branches, each branch being once or twice forked. The final branches bear the green conidial fructifications, which measure  $8$  to  $10\ \mu$ . The conidiospores are elliptical, almost hyaline, aggregated without mucilage to form a ball, and measure  $2.5$  to  $3\ \mu$  by  $3$  to  $4\ \mu$ .

Unnamed species of *Trichoderma* were found by Otto<sup>55</sup> and by Heukelekian<sup>3</sup> to be among the most vigorous cellulose-decomposing fungi met with in soil.

Subdivision : *Botrytideae*.*Sporotrichum bombycinum*, Corda.Syn. : *Capillaria bombycina*, Corda.*Sporotricha bombycina*, Rabenhorst.

This species was found by van Iterson, jr.<sup>40</sup>, to possess slight cellulose-decomposing properties.

The growth consists of a white, matted, cotton wool-like mycelium of branched hyaline hyphae. The ellipsoidal conidia are white, measuring 3 to 4  $\mu$  by 4 to 5  $\mu$ , and may occasionally be pointed.

*Sporotrichum griseolum*, Oudemans.

Is also stated by van Iterson, jr.<sup>40</sup>, to possess weak cellulose-decomposing properties.

No description of this fungus appears in any of Oudemans's published papers, and the name should probably be regarded as *nomen nudum*.

*Sporotrichum roseolum*, Oudemans and Beijerinck.

Like the two preceding species, this was shown to be capable of slight cellulose decomposition in van Iterson, jr.'s<sup>40</sup> experiments.

The widely spread mycelium is faintly rose-coloured and consists of sparingly septate, delicate, irregularly branched, creeping hyphae. The unbranched or branched side branches serve as conidiophores. The conidia are formed terminally and are spherical to egg-shaped, hyaline when observed singly, and pale rose colour when observed in mass. They measure 3 to 5  $\mu$  by 3 to 4  $\mu$ .

*Botrytis Bassiana*, Balsamo.Syn. : *Stachylidium Bassianum*, Montagne.

This was found by Miyoshi<sup>50</sup> to be capable of penetrating membranes containing cellulose, partly by mechanical pressure and partly by enzymatic activity.

The mycelium forms a felted white layer from which the white conidiophores arise. They are unbranched or branched; in the latter case the short branches are arranged in forks. The spherical conidia measure 2 to 3  $\mu$  in diameter and are arranged in clusters at the tips of the branches.

*Botrytis tenella*, Saccardo.

This was found by Miyoshi<sup>59</sup> to behave in a similar manner to the preceding species towards membranes containing cellulose.

The white mycelial growth is somewhat firmer than that of the previous species and consists of septate branched hyphae measuring 1.5 to 2  $\mu$  in diameter. The erect conidiophores are irregularly branched, sparsely septate, and hyaline. The egg-shaped conidia measure 1.5 to 2  $\mu$  by 2.5 to 3  $\mu$ , are hyaline, and often possess oil globules. They are formed in clusters of two or three groups.

Subdivision: *Verticilleae*.

*Acrostalagmus cinnabarinus*, Corda.

Syn.: *Botrytis cinnabarina*, Fries.

This was studied by Sée<sup>41</sup>, who found that it produced on paper yellowish red spots surrounded by a yellowish or slightly orange zone (see Table II, p. 291).

The growth is composed of a matted layer of septate hyphae and erect septate conidiophores with whorls of four or five branches, which again possess whorls of four secondary branches. The latter measure 12 to 14  $\mu$  by 3 to 4  $\mu$ . The conidia are ellipsoidal, 3 to 4  $\mu$  long by 1.5  $\mu$  broad. They are rose-coloured and later hyaline. They contain no oil drops.

Oudemans and Koning<sup>10</sup> isolated a species closely related to this type from humus, and they named it *Acrostalagmus cinnabarinus*, Corda, var. *nana*, Oudemans. They give the following description of this variety:

The growth consists of orange or red tufts of creeping, branching, septate hyphae. The septate conidiophores possess two or three series of opposite unicellular branches, surmounted by three verticillate skittle-shaped cells, each of which bears a mass of elliptical or oblong conidia, 3.5  $\mu$  by 5 to 8  $\mu$ , adhering to one another by a mucous liquid. All parts of the fungus are tinted a very pale pink. The variety differs from the type species in its smaller dimensions, its unicellular opposite, not verticillate, branches divided into three, not four, secondary branches. The conidia, on the other hand, are larger (Oudemans and Koning<sup>10</sup>).

*Spicaria elegans*, Corda, var. *flava*, Sée.

Occurred occasionally on paper in Sée's<sup>41</sup> investigations. It produces light brown or coffee-coloured spots surrounded

by yellowish or light brown zones caused by the secretion of a pigment (see Table II, p. 291).

The velvety white growth of the type species consists of hyaline septate creeping hyphae. The erect conidiophores are septate and covered with delicate short hairs. Towards the tip they bear several whorls of two or more branches, each of which bears a whorl of three or four branchlets at the end. The ovoid to spindle-shaped conidia are formed in long chains from these branchlets. They are hyaline and measure  $3.5$  to  $4\ \mu$  by  $4$  to  $5\ \mu$ .

The variety studied by Sée differs from the type species in the absence of the hairy covering of the conidiophore and in being yellow to brown in colour.

*Spicaria simplicissima*, Jensen.

This species was reported by Scales<sup>44</sup> to be capable of decomposing cellulose in the presence of inorganic nitrogen.

The species quoted by Scales appears to be unknown to Rabenhorst, who describes *Spicaria simplicissima*, Oudemans, isolated by Koning from humus, as forming a circular growth of delicate creeping, septate, hyaline, dichotomously branched hyphae. The growth is coloured cream-yellow to dirty grey in patches. Occasionally patches of purple are found. The erect conidiophores attain a height of  $40\ \mu$  and are septate, hyaline, and generally unbranched, with a whorl of three unseptate hyaline branchlets at the tip. These branchlets measure  $8$  by  $12\ \mu$ . The spherical conidia are formed in short chains of two or three at the tip of the branchlets.

*Verticillium latericium*, Berkeley.

This was found by Heller<sup>30</sup> to possess slight cellulose-decomposing properties.

The growth is woolly, velvety, and vividly red to cinnabar-red in colour. The closely set cinnabar-red conidiophores possess several whorls of secondary branches, each of which again possesses a whorl of three or four branchlets. The conidia are elliptical, tapering towards the ends,  $2.5$  to  $3\ \mu$  by  $4$  to  $6\ \mu$  in size and are light cinnabar-red.

Sub-family: *Hyalodidymae*.

*Trichothecium roseum*, Link.

Sée<sup>41</sup> occasionally found this species, under the name of *Cephalothecium roseum*, Corda, occurring on mildewed paper,

on which it produced salmon pink to rose-coloured spots, surrounded by yellowish to slightly pink zones of pigment secreted by the fungus (see Table II, p. 291). Heller<sup>30</sup> reports it to be capable of decomposing cellulose, although Schellenberg<sup>6</sup> previously found it unable to do so.

A description of this species is given under Group A (Chapter V).

Sub-family: *Hyalophragmiae*.

*Blastotrichum*, Corda.

Syn.: *Anodotrichum*, Corda.

This genus appears to contain several species which decompose dead vegetable matter. Direct evidence of cellulose decomposition within this group has so far been obtained in the case of an unnamed species by Otto<sup>55</sup>, and by van Iterson, jr.<sup>40</sup>, in the case of *Blastotrichum puccinioides*, Preuss (syn.: *Mycogone puccinioides*, Saccardo), a parasitic species living on *Russula rubra* and *Russula livide*.

The genus forms a layer of mycelium composed of creeping, branched, septate hyphae. The conidia-bearing branches are erect, irregularly branched and septate. The conidia occur singly and are elliptical with rounded or pointed ends. They have two or more septa when ripe, the spore wall being constricted at the regions of septation. The spores are hyaline or light in colour.

Family: *Dematiaceae*. Sub-family: *Phaeosporae*.

Subdivision: *Periconaeae*.

*Stachybotrys alternans*, Bonorden.

Van Iterson, jr.<sup>40</sup>, found that this species had fairly marked cellulose-destroying properties.

The sterile hyphae are creeping and show scorpioid dichotomous branching. They are often papillate, are sparsely septate, dark brown in colour, and measure 3 to 5  $\mu$  in diameter. The erect slender conidiophores are grey to hyaline, usually unbranched, and measure 3.5  $\mu$  in diameter. At the tip they bear a number of closely set ovoid or club-shaped, grey or hyaline sterigmata which measure 4 to 5  $\mu$  by 10  $\mu$ . At their tips are formed the egg-shaped papillate black conidia, measuring 5 to 7.5  $\mu$  by 8 to 12  $\mu$ . They occasionally contain two oil globules.



*Stachybotrys atra*, Corda.

This species was reported to be present on mildewed cotton goods by Davis, Dreyfus, and Holland<sup>47</sup>. Sée<sup>41</sup> found it commonly represented on mildewed paper, on which it produced black or greenish-black spots surrounded by greenish-grey to brown zones due to a pigment secreted by the mycelium of the fungus (see Table II, p. 291).

The mycelium forms a delicate black growth. The hyphae are dichotomously branched and sparsely septate. The individual hyphae appear yellowish-green in colour. Conidiophores are formed as branches, with spindle-shaped, almost hyaline sterigmata. The conidia are egg-shaped, smooth, brown, 8 to 9  $\mu$  in length and contain two oil globules. The presence of a septum has been reported.

An unnamed species of *Stachybotrys* was reported by Otto<sup>55</sup> to be capable of decomposing cellulose.

Subdivision: *Toruleae*.*Torula chartarum*, Link.

Syn.: *Stilbospora chartarum*, Ehrenberg.

*Oidium chartarum*, Link.

*Sporotrichum chartaceum*, Persoon.

*Torula chartarum*, Corda.

This was found by Sée<sup>41</sup> to be an inhabitant of mildewed paper, on which it produced black spots (see Table II, p. 291).

The growth consists of an extensive layer of creeping, branched, hyaline, septate mycelium. The conidiophores arise as short hyaline branches of the mycelium, gradually merging into long curved or straight chains of conidia. The conidia are egg-shaped, smooth, and brown, giving a blackish appearance to the growth. The spores measure 5 to 6  $\mu$  by 8 to 9  $\mu$ .

*Torula ligniperda*, Willkomm.

Syn.: *Zenodochus ligniperda*, Willkomm.

*Torula ligniperda*, Saccardo.

The spores of this species were found scattered deeply in the wood of logs of white ash (*Fraxinus americana*) and yellow poplar (*Liriodendron tulipifera*) by Siggers<sup>103</sup>. Though the cell walls of the attacked wood are seldom perforated by

the mycelium of this species, Siggers nevertheless found that heavily infected logs had decreased in strength.

On straw used for paper making, Haas<sup>91</sup> found this or a related species producing blackish papillae.

The creeping, vegetative hyphae infest the wood cells. They are greyish-black, sparingly branched, and sparsely septate. The chains of conidia are formed at the tips of the hyphae or on branches, and in either case inside the wood cells. The chains consist of 6 to 12 conidia, which are spherical to ellipsoidal, smooth, black, and often contain an oil globule. The conidia measure 8 by 10  $\mu$ .

The species of the genus *Torula* appear to occur chiefly on decaying plant material, but only in the two cases mentioned has direct evidence been obtained of their association with cellulose decomposition.

#### Subdivision: *Trichosporieae*.

##### *Trichosporium*, Fries.

Here again the genus is widely distributed on decaying plant material. A species of *Trichosporium* was found by Heukelekian<sup>3</sup> to cause decomposition of cellulose in soil.

The hyphae are creeping, irregularly branched, and light or dark brown in colour. The conidia are formed at the tips of hyphae, or of branches, or on the sides of the hyphae. They are spherical or egg-shaped. They are also smooth or somewhat rough, brown or occasionally hyaline. Like *Sporotrichum*, which it resembles, the genus is little known.

#### Sub-family: *Phaeodidymae*.

##### Subdivision: *Bisporeae*.

##### *Bispora monilioides*, Corda.

Tuzson<sup>63</sup> states that this attacks freshly felled logs of beechwood, causing a white rot.

The growth forms an extensive, glistening, blackish-brown, dusty layer. The mycelium, which is transparent and smoke-grey, is little branched and about 3.5  $\mu$  in diameter. The conidia-bearing side branches are short and almost skittle-shaped. The conidia are short and fusiform, with a thick septum, each cell showing an

oil globule. The spores are not constricted at the place of septation, are smoke-black in colour, and measure 6 to 7  $\mu$  by 19 to 22  $\mu$ . The conidia are formed in chains of three or more, one chain on each conidiophore.

*Dicoccum asperum*, Corda.

Syn. : *Sporidesmium asperum*, Corda.  
*Trichocladium asperum*, Harz.

This species, under the name of *Trichocladium asperum*, was found by van Iterson, jr.<sup>49</sup> to be capable of decomposing 9 per cent. of the cellulose exposed to destruction in 40 days. This rate was considered by him to be sufficient to rank the species as a powerful cellulose decomposer.

It forms a black granular spreading growth on the surface of the substratum, and is composed of delicate, creeping, branched, hyaline or yellowish hyphae, which are usually sparsely septate. The papillate conidia are borne singly on short side branches. When young they are hyaline, but later become yellowish-brown to black. They are oval and usually consist of two cells, the spore wall being constricted at the place of septation. In the young spores the lower cell is frequently pointed and smaller than the upper. The whole spore measures 10 to 13  $\mu$  by 18 to 26  $\mu$ .

#### Subdivision : *Cladosporiaceae*.

*Cladosporium herbarum*, Link.

Several workers have found this species on decaying cellulose. Sée<sup>41</sup> reported its occurrence on paper, Davis, Dreyfus, and Holland<sup>47</sup> found it on mildewed cotton goods, while Hauman<sup>58</sup>, and later Ruschmann<sup>8</sup>, observed it on dew-retted flax fibres. Van Iterson, jr.<sup>49</sup>, showed that it destroyed cellulose to a moderate extent, and Kosin<sup>104</sup> also found it active in this respect. Schellenberg<sup>6</sup> appears to be the only investigator who could obtain no positive evidence of its action on cellulose.

Unnamed *Cladosporium* species were reported present on mildewed cotton goods by Levine and Veitch<sup>57</sup> and by Bright, Morris, and Sumners<sup>52</sup>, and on wood-pulp by Barnes<sup>105</sup>.

A description of the morphology of *Cladosporium herbarum* is given under Group A (Chapter V).

Sub-family : *Phaeodictyae*.

Subdivision : *Macrosporiceae*.

*Stemphylium botryosum*, Wallroth.

Syn. : (according to Sée) *Urocladium botrytis*, Preuss.

This was isolated by Oudemans and Koning<sup>10</sup> from humus. A variety of the species was obtained by Sée<sup>41</sup> from mildewed paper.

The creeping hyphae spread widely over the substratum. When young they are thin, irregularly branched, hyaline, and unseptate. On ageing they become brownish, beaded, and septate. The ordinary branches are short, beaded, branched or unbranched, hyaline or coloured, and frequently forked at the tip. Each bears one conidium on a short pedicel. The conidium is spherical to elliptical and is divided horizontally into two to six cells of which one or more may possess a vertical or oblique septum. The colour of the conidium is fawn to blackish-brown. The conidia measure 16 to 20  $\mu$  by 25 to 40  $\mu$ , and when old their surface is finely stippled (Oudemans and Koning<sup>10</sup>).

*Stemphylium botryosum*, Wallroth, var. *domesticum*, Saccardo.

This, or a closely related variety, was found by Sée<sup>41</sup> to produce deep brown spots on paper (see Table II, p. 291).

In this variety the conidia are inverted egg-shaped, and possess three septa. The conidia are smoke-coloured and the mycelium is hyaline.

*Stemphylium graminis*, Corda.

Syn. : *Soredospora graminis*, Corda.

*Stemphylium graminis*, Bonorden.

This and the other species of *Stemphylium* enumerated below were found by Sée<sup>41</sup> on mildewed paper, on which they produced black stains (see Table II, p. 291).

The growth consists of an extended, almost paper-like black mycelium composed of extensively branched, beaded, ochre-coloured transparent hyphae. The spores are brownish-black, 10 to 30  $\mu$  in diameter, and possess transverse and vertical septa.

*Stemphylium macrosporoides*, Berkeley.

Syn. : *Epochium macrosporoides*, Berkeley et Broome.

*Stemphylium macrosporoides*, Saccardo.

In addition to Sée<sup>41</sup>, van Iterson, jr.<sup>40</sup>, has studied this species in its relation to cellulose, of which he found it to be a very active decomposer. Van Iterson also emphasizes that there is a great similarity between this species, *Mycogone puccinioides* and *Trichocladium asperum*.

The growth consists of an extensive, dark-coloured mycelium. The hyphae are delicate, irregularly branched, hyaline, septate, and interwoven. Side branches bear the spherical to mulberry-shaped conidia, which are hyaline and unicellular when young, but later become chestnut-brown and divided into four cells. By the septation the lowest cell may become slightly swollen and serve as a pedicel for the remaining three. The spores measure 14 by 25  $\mu$ .

*Stemphylium piriforme*, Bonorden.

The mycelial layers are black and fairly loose. The hyphae are extensively branched, creeping, septate, and smoke-coloured. The greyish-black conidia are formed at the tips of the hyphal branches. They are inverted pear-shaped or ovoid, and have three or four septa arranged muriformly. The walls of the conidia are constricted at the points of septation. The conidia measure 12 to 15  $\mu$  by 25 to 30  $\mu$ .

*Stemphylium verruculosum*, Zimmermann.

Syn.: *Macrosporium verruculosum*, Zimmermann.

*Stemphylium verruculosum*, Saccardo.

The growth consists of a widespread olive-coloured layer of curved, branched, septate hyphae, 22  $\mu$  in diameter. The conidia are egg-shaped or elliptical, and possess two or three horizontal septa in addition to vertical, thus giving the spore wall a muriform appearance. The conidia are brown, opaque when ripe, and papillate. They measure 11 to 13.5  $\mu$  by 17.5 to 22  $\mu$ .

Unnamed species of *Stemphylium* have been studied in their relationship to cellulose by Otto<sup>55</sup>, Broughton Alcock<sup>106</sup>, and Ramsbottom<sup>107</sup>. Most species of the genus are probably capable of decomposing cellulose.

#### Subdivision: *Alternarieae*.

The genus *Alternaria* contains several species which have been recorded as present on decaying dead plant material. Unnamed species have also been observed on mildewed cotton goods by Levine and Veitch<sup>67</sup> and by Bright, Morris, and

Summers<sup>52</sup>. The undermentioned species have been definitely associated with the destruction of cellulose.

*Alternaria chartarum*, Preuss.

Was found by Sée<sup>41</sup> on mildewed paper, on which it produces dark grey to black spots through the spread of its dark-coloured mycelium (see Table II, p. 291).

The mycelium is widely spreading and brown in colour, turning black with age. It consists of creeping or erect, branched, septate hyphae. The conidia are spherical to elliptical and taper to a short neck at one end, by which they are connected to succeeding conidia to form a chain. They are septate, muriform, and brown to greenish-black.

*Alternaria humicola*, Oudemans.

This species forms a yellowish to brownish pigment and was observed by Sée<sup>41</sup> on mildewed paper as deep brown to almost black spots, sometimes surrounded by a slight yellowish or brownish zone (see Table II, p. 291). Oudemans and Koning<sup>10</sup> isolated the species from decayed leaves.

The ripe mycelium forms a circular greenish-black layer. The branches of the articulate conidiophores are successively shorter towards the top. They are hyaline and measure 3 to 5  $\mu$  in diameter. The conidia are irregularly shaped, being cylindrical, inverted club-shaped or bottle-shaped. When young they are hyaline, but later become brown to greenish-black. They possess three to seven muriform septa. The walls are but slightly, if at all, constricted at the points of septation. When ripe their surface is punctate and rough. They measure anything up to 16 by 50  $\mu$ .

*Alternaria polymorpha*, Planchon.

This species forms deep brown spots on mildewed paper (Sée<sup>41</sup>) (see Table II, p. 291). The fungus does not, however, produce a soluble pigment.

The mycelium is olive-green, septate, and frequently contains oil globules. The fertile branches are almost straight, septate, with fragile, sometimes branched, chains of four to five spores. The deep brown ripe spores are irregularly pyriform and divided by transverse and longitudinal septa into six to twelve cells. The spore walls are thick, with constrictions and a lighter coloured tip. The average measurements of the spores are 20 by 10  $\mu$ . On

germination each spore gives rise to one to four tubes. The connecting link between the individual spores is whitish, later brown. It measures 5 to 12  $\mu$  by 3 to 5  $\mu$ .

A pinkish yeast-like growth may be formed. These cells are surrounded by a thin mucilaginous layer and contain refractive globules. When older the cells are often divided by a transverse septum. They measure 2 to 12  $\mu$ .

Pycnidia are formed. They develop rapidly, in three to four days, and vary in size up to 250 by 350  $\mu$ . Stylospores are liberated from an orifice in the pycnidium aggregated in a mucilaginous substance, and form on unrolling a ribbon-like mass of spores measuring 4 by 3  $\mu$ . These grow into yeast-forms recalling the 'conidia' of *Dematiium*. When fully developed they measure 10 by 8  $\mu$ . They contain refractive bodies, and are white, later becoming brown (Sée<sup>41</sup>).

*Alternaria varians*, Planchon.

This species forms brown to almost black spots on mildewed paper (Sée<sup>41</sup>), and, like the preceding species, does not secrete a soluble pigment (see Table II, p. 291).

The growth on carrot is grey to greyish-brown, consisting of brown, septate, branched mycelium, and branched or unbranched conidiophores. The hyphae measure from 2 to 5  $\mu$  in diameter and frequently interlace and anastomose. The spores are blackish-brown, usually pyriform. They are usually muriformly septate, thickened, and constricted when ripe. They often terminate in a white tip, and measure 15 to 18  $\mu$  by 23 to 32  $\mu$  or more.

Other smaller brownish-black and thicker walled, often opaque, spores are also formed. They are divided by transverse septa into three to four cells, and are constricted. They contain oil drops. The fragile chains are long and the spores are separated by small hyaline links. On germination the spores give rise to two to three tubes, the hyphae thus formed showing a tendency to anastomose (Sée<sup>41</sup>).

Family: *Stilbaceae*. Sub-family: *Phaeostilbaceae*.

Subdivision: *Amerosporae*.

*Stysanus stemonites*, Persoon.

Syn.: *Periconia stemonites*, Persoon.

*Cephalotrichum stemonites*, Nees.

*Isaria stemonites*, Sprengel.

Was found by Oudemans and Koning<sup>10</sup> in humus and by Sée<sup>41</sup> on mildewed paper, on which it produced deep brown,

almost black, spots (see Table II, p. 291). Osborn<sup>56</sup> reports the presence of an unnamed species of *Stysanus* on mildewed cloth.

The coremia occur in clusters. They form thin, unbranched, dark brown stems composed of parallel, septate, green-brown hyphae which terminate in cylindrical heads. The ovoid to lemon-shaped, transparent, blue green conidia are formed in chains and measure 4 to 5  $\mu$  by 6 to 8  $\mu$ .

This is the only species of the genus *Stysanus* which is reported to be capable of decomposing cellulose. Many others occurring on decaying plant material are undoubtedly also able to do so.

Family: *Tuberculariaceae*. Sub-family: *Tuberculariaceae  
muucedineae*.

Subdivision: *Phragmosporae*.

*Fusarium* spp.

Species of *Fusarium* have frequently been reported as capable of destroying cellulose, for instance, in soil by Heukelekian<sup>3</sup>. They have also been found on fabrics by Osborn<sup>56</sup>, Armstead and Harland<sup>51</sup>, and Bright, Morris, and Summers<sup>52</sup>. Sée<sup>41</sup> studied two species from mildewed paper, of which one caused white patches and the other red, brown, or deep brown spots.

A number of parasitic species of *Fusarium* have been studied by Appel and Wollenweber<sup>108</sup>, and of such forms Heller<sup>30</sup> found several, among them *Fusarium lolii*, *Fusarium rubiginosum*, and *Fusarium subulatum*, to be capable of decomposing cellulose. A very large number of the species of this genus participate in the decay of dead plant tissues and will undoubtedly eventually be found capable of decomposing hemicelluloses and cellulose.

Sub-family: *Tuberculariaceae dematieae*.

Subdivision: *Amerosporae*.

*Epicoccum purpurascens*, Ehrenberg.

Syn.: *Epicoccum vulgare*, Corda.



This species was described by van Iterson, jr.<sup>40</sup>, as a powerful cellulose decomposer.

The fructifications are brownish-black, spherical, and measure 120 to 150  $\mu$  in diameter. They are aggregated to form longitudinal layers 2 to 3 mms. in length, and are superimposed on a purplish-coloured base. The conidia are almost spherical, at first yellowish and later brown, reticulate and papillate, tapering at the base to a short hyaline stem. They measure 12 to 22  $\mu$  in diameter.

Many species of this genus occur on decaying vegetable tissues.

## LITERATURE

### CHAPTERS V AND VI

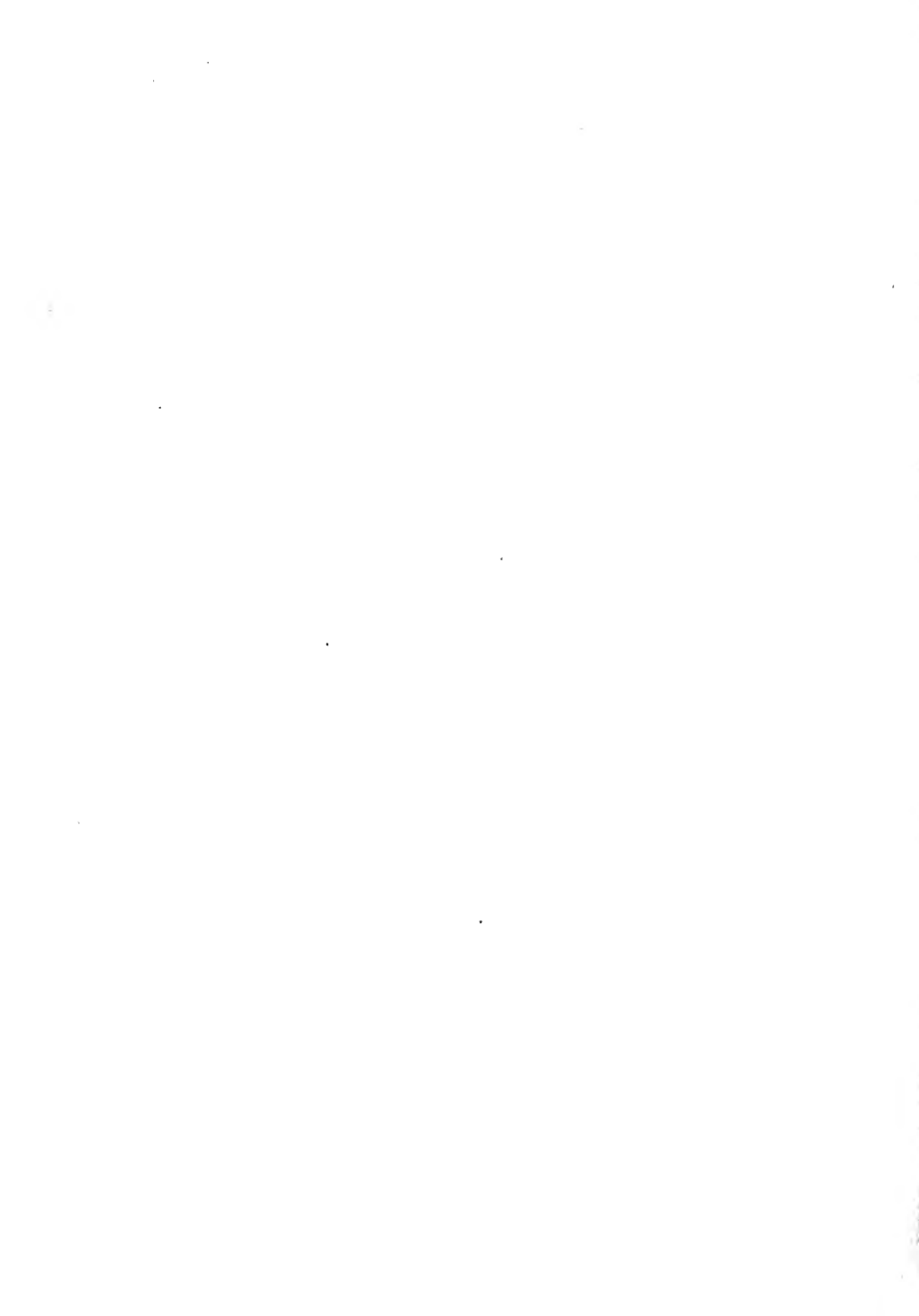
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PART THREE

MICROBIOLOGICAL DECOMPOSITION PROCESSES  
OF GUMS, PECTIN, HEMICELLULOSES, AND  
CELLULOSE



## CHAPTER VII

### GUMS AND PECTIN

THE inadequacy of existing knowledge of the micro-organisms which decompose gums has already been referred to. Even less complete is the exploration of the successive chemical stages of decomposition of these substances through the action of microbiological enzymes. From analogy with other similar biological processes, as well as from the changes brought about by the chemical hydrolysis of gums, it is probable that, on being decomposed by micro-organisms, gums first become hydrolysed into their component carbohydrates, thereby losing their adhesive properties. Subsequently acids are probably formed, though their nature has hardly been explored. Whether alcohol or ketones are also produced has not been ascertained. In view of the comparative insignificance of the gums from an industrial point of view it is not likely that this lack of definite information will be speedily remedied, unless it is shown, as has already been suggested by Omelianski<sup>1</sup>, that some gums constitute favourable sources of carbohydrates for the development of micro-organisms capable of decomposing cellulose.

**Microbiological decomposition of pectin.** The plea of industrial unimportance can hardly be advanced as an excuse for the almost equally fragmentary knowledge of the biochemical changes involved in the microbiological decomposition of pectin. Even the most recent papers on the important subject of the retting of flax and hemp, in which a decomposition of pectin is involved, pay comparatively little attention to the question of the decomposition products of the pectin. The most exhaustive information in this respect is supplied by Störmer<sup>2</sup>, who identified acetic and butyric acids, besides traces of lactic and valeric acids, as decomposition products of his

anaerobic retting organism *Plectridium pectinovorum*, and found that the gas evolved during the breakdown of pectin by this organism consisted of hydrogen and carbon dioxide. Further light may be thrown on the biochemical changes caused by anaerobic pectin-decomposing bacteria if it be assumed that the group of micro-organisms already referred to as *Bac. amylobacter* can be regarded as the most characteristic anaerobic pectin decomposers, and that the breakdown of pectin by this group proceeds on the same lines as the decomposition of starch and other carbohydrates.

In his extensive study of the *Bac. amylobacter* group, Bredemann<sup>3</sup> endeavoured to establish the nature of the decomposition products formed by this organism in the fermentation of starch. A fuller account of these changes, however, is given by Reilly and his collaborators<sup>4</sup> in their investigation of the products of the acetone: *n*-butyl alcohol fermentation of carbohydrate material. Before describing this investigation, it should perhaps again be emphasized that the group of *Bac. amylobacter*, as established by Bredemann<sup>3</sup>, comprises a variety of species differing in several respects, as for example in their capacity for converting the volatile acids produced by them in the decomposition of carbohydrates into alcohols and ketones. Some species possess this property to a very marked degree, whereas others lack it almost entirely. This may readily be shown by repeating Mitscherlich's<sup>5</sup> experiments on the isolation of pectin-decomposing 'ferments' from potatoes.

The type of *Bac. amylobacter* with which Reilly and his collaborators worked belonged to the group yielding little organic acid and large amounts of alcohol and acetone. The yields of a typical fermentation of starch by this organism are given as follows:

100 grms. of starch yielded :	{	10.77 grs. of acetone.
		25.07 grs. of <i>n</i> -butyl alcohol.
		62.61 grs. of carbon dioxide.
		1.60 grs. of hydrogen.
		1.80 grs. of residual acids.

The residual acids consisted of acetic and butyric acid in the proportions of 1.0 to 0.25. The low proportion of butyric to acetic acid must no doubt be regarded as a result of

the differences in the extent to which the two acids were being gradually reduced to *n*-butyl alcohol and acetone, a larger amount of butyric acid being converted into *n*-butyl alcohol than acetic acid into acetone. When the proportion of acids was determined at an earlier stage in the fermentation, it was found that approximately 1 part of acetic acid was formed to 1.25 parts of butyric acid, a proportion which probably corresponds more closely to those met with in retting liquors.

In addition to acetic and butyric acids, Reilly and his collaborators obtained evidence of the presence of yet another acid, not readily volatile; this, however, they were unable to identify.

Thus, in the anaerobic fermentation of pectin the following substances are likely to be met with: carbon dioxide and hydrogen in the approximate proportions of two volumes of carbon dioxide to one volume of hydrogen, acetic and butyric acids, alcohols, such as ethyl and *n*-butyl alcohol, acetone, and possibly esters. The presence of several of these substances has been reported in the retting liquors of the various anaerobic retting processes.

The chemical changes which take place when pectins are broken down by aerobic micro-organisms are unknown, in spite of the fact that a promising aerobic retting process for the preparation of hemp and flax fibres is being worked industrially (Rossi<sup>6</sup>).

Apart from the importance of the question of the natural destruction of pectin in the rotting of fruits, a subject which is dealt with in text-books on plant pathology, the microbiological decomposition of this substance is of considerable importance in two directions, in the retting of plant tissues for the isolation of fibres, and in the preparation of starch from potatoes and wheat.

✓ **Retting of fibre plants.** The art of retting plant tissues for the isolation of fibres has been carried out in many countries from time immemorial, and highly developed methods for the retting of flax existed in countries such as Egypt from the earliest dynastic times. Here conditions were undoubtedly



particularly favourable, the river Nile with its slow-moving and comparatively warm and soft waters forming an ideal basis for the elaboration of a natural retting process. A different method was probably adopted by the inhabitants of the Swiss lake dwellings, who, it appears from the remains found, were well acquainted with the art of retting. Here the process was probably carried out in stagnant water. Both of these methods, the retting in stagnant and in slow-flowing water, were the only two anaerobic retting processes in use up to the middle of the nineteenth century. They are still extensively used, the former in Ireland and Italy, the latter in Holland, Belgium, and Germany.

In both cases the principles of the retting are the same and may be divided into three stages, a physical, a biological, and a mechanical stage. On submerging the dried plant tissues such as flax straw in water, the latter causes the straw to swell. At the same time the air is expelled from the tissues and a number of substances, carbohydrates, glucosides, tannin, nitrogen compounds and colouring matter, are brought into solution. This represents the physical stage of the retting. The biological stage may be divided into two, the preliminary and the principal. In the former, a great variety of micro-organisms develops at the expense of the extracted substances, thereby creating anaerobic conditions in the water and paving the way for the anaerobic pectin-decomposing bacteria, which are normally found in all arable soils and thence become transferred to the flax straw by wind and splashing rain. In the principal biological phase the anaerobic pectin decomposers dissolve the middle lamellae of the parenchymatous tissues, separating the fibre bundles from the wood and the cortex. Fig. 8 illustrates the morphology of a typical flax stem: it is taken from a recent paper by Eyre and Nodder<sup>7</sup>.

Where the fibres are contained in leaves the morphology is naturally different. The principle that the fibres are embedded in parenchymatous tissues is, however, the same. By the resolution of the middle lamellae of the phloem and the cortex during the principal biological phase the fibre bundles become

separated and may be collected more or less free from other tissues during the subsequent mechanical phase. In this, the retted fibre tissues are allowed to dry and are then passed through specially designed machinery which eliminates wood and cortical residues.

From this broad outline it is now necessary to proceed to a more detailed account of the first three stages of the retting process in order to obtain an insight into the microbiology of retting.

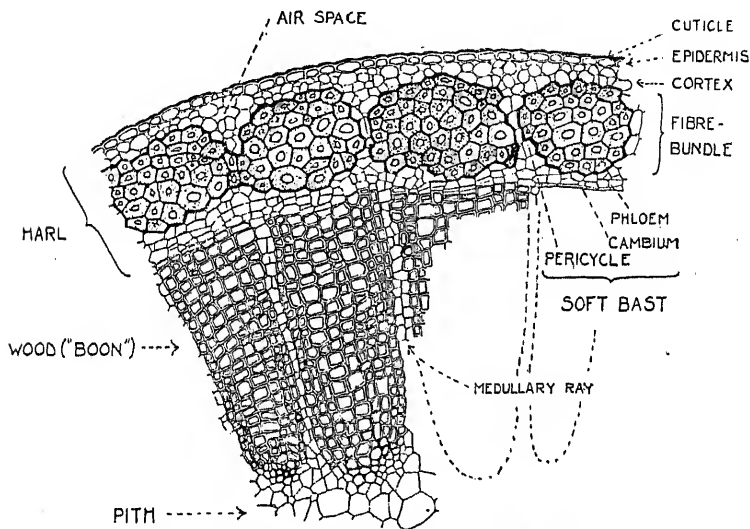


FIG. 8. Diagrammatic cross-section of a flax stem.  
(From Eyre and Nodder, in the *Journal of the Textile Institute*.)

Though micro-organisms play a comparatively insignificant role in the physical phase, this stage is nevertheless of immense importance from the biological point of view, since the microflora subsequently developing in the retting water will very largely depend not only on the nature of the substances extracted, but equally on the concentration in which they are present in the retting water. The entry of water into the submerged tissues will not only replace any enclosed air, as has already been mentioned, but will also cause the tissues to swell and the cortex to burst. Orifices are thereby formed

through which the retting organisms can enter (Müller and Tobler<sup>8</sup>). The swelling of the tissues will also materially favour the removal into the surrounding liquid of decomposition products formed during retting, and will thereby assist in maintaining an homogeneous ret. The extraction of the tissues during the physical stage removes about 12 per cent. of the contents of flax straw (Ruschmann<sup>9</sup>). These substances give the water a yellow or brown colour, depending largely on the colour of the straw used. At 20° C. the physical phase may be completed in from six to twelve hours.

Very early during the preliminary biological stage, sometimes within twenty-four hours of the immersion of the tissues, the surrounding liquid becomes cloudy through the development of micro-organisms. Though a detailed study of this flora has not been made, the following types have been reported present on flax fibres or in the retting liquor in which flax was being retted under anaerobic conditions: *Bac. mesentericus*; *Bac. subtilis*; *Bac. mycoides*; *Oidium lactis*; *Cladosporium herbarum*; *Torula* species; lactic acid-producing bacteria; denitrifying bacteria; and *Bact. coli*. This last type was considered identical with *Bact. coli commune*, Escherich, an inhabitant of the intestine. More probably, however, it belongs to that large group of bacteria resembling *Bact. coli commune* which is continually met with in soil, in water, and on leaves, and which differs from the typical *Bact. coli commune* in its inability to produce indol. Hauman's<sup>10</sup> statement that he isolated two different types of *Bact. coli* supports this point of view.

Though several investigators, among them Hauman<sup>10</sup>, Behrens<sup>11</sup>, and Beijerinck and van Delden<sup>12</sup>, have reported that some at least of the micro-organisms mentioned above are capable of decomposing pectin, there can be no doubt that the main function of this flora during the anaerobic retting must be a different one, since only a small proportion of the available pectin is decomposed during the preliminary biological phase of the ret (Eyre and Nodder). As they are either carbohydrate-decomposing or denitrifying forms, and as they all develop either aerobically or at least facultatively anaerobically, it is reasonable to assume that they play a

leading part in the decomposition of the substances extracted from the fibre tissues, and in so doing establish anaerobic conditions in the liquid, thereby preparing the way for the anaerobic *Bac. amylobacter*. The importance of this latter function has repeatedly been emphasized and perhaps even overrated, since it is known from Tarozzi's<sup>13</sup> investigations that anaerobic bacteria can develop in the presence of oxygen, when certain solid substances, such as living tissues, are available in the medium. Tobler<sup>14</sup> has confirmed Tarozzi's observation in his study of the anaerobic retting organism *Bac. felsineus*, for the cultivation of which Carbone<sup>15</sup> recommends the addition of a culture of a saccharomycete, *Saccharomyces ellipsoideus*, in order to establish anaerobic conditions. Tobler found that *Bac. felsineus* grows well in potato mashes in the presence of oxygen and in the absence of the saccharomycete, presumably because of the presence in the mash of solid particles of potato tissue. The assumption that the establishment of anaerobic conditions in the retting liquor is of minor importance for the development of *Bac. amylobacter* is supported by yet another observation. Where the liquor containing the extracted substances is replaced by fresh water, the development of *Bac. amylobacter* proceeds normally, while the activity of the secondary microflora becomes greatly restricted. Only when the retting liquor is very violently aerated does the development of *Bac. amylobacter* appear to be retarded or suppressed (Ruschmann<sup>16</sup>).

When the anaerobic retting is carried out in stagnant or in very slowly-flowing water, the secondary microflora gradually forms a thick white or greyish layer on the surface of the liquid. This layer consists of the mycelium of fungi, such as *Oidium lactis*, and of the cells of *Bac. mesentericus* and the allied spore-forming soil organisms.

The breakdown of the carbohydrates and of the nitrogen compounds extracted from the fibre tissues results in the formation of acids and gas. The acid produced consists partly of lactic acid (Beijerinck and van Delden<sup>12</sup>) and possibly also of butyric acid. The presence of lactic acid is important since this acid has been shown to interfere seriously

with the development of *Bac. amylobacter* (Thaysen<sup>17</sup>). The production of lactic acid as well as of other inhibitory substances during the preliminary biological phase makes it essential to use a comparatively large proportion of water in order to prevent a serious accumulation of these products. The proportion of water to tissues depends to a large extent on the material being retted. In the case of flax straw it may be twenty to one (Krais<sup>18</sup>).

As already pointed out, the gas produced from the carbohydrates consists normally of a mixture of carbon dioxide and hydrogen. Where nitrates and other reducible nitro-compounds are available, these may possibly be reduced to free nitrogen (Behrens<sup>19</sup>). The production of free nitrogen during the retting of flax was observed by Hodges<sup>20</sup> in 1854. The evolution of gas during this part of the retting process causes the liquor to froth considerably. As this frothing subsides, towards the end of the preliminary biological stage, the whitish film already mentioned develops and soon covers the surface of the retting liquor. The duration of the preliminary biological stage will depend on a number of factors, such as the temperature at which the ret is being conducted, and on the concentration of the substances extracted. At a temperature of 20° C. it may continue for 100 hours, or possibly more.

Though the development of the pectin-decomposing *Bac. amylobacter* has undoubtedly started during the preliminary biological phase, it is during the subsequent stage that its activity becomes most marked. A microscopic examination of the tissues undergoing retting reveals, during the principal biological stage, a large number of the characteristic *Clostridium* or *Plectridium* forms, accumulated between the cells of the phloem and the cortex, often embedded in a slimy substance. The decomposition of the pectin of the middle lamellae and possibly of the cell walls of the cambium and the phloem gives rise to the evolution of carbon dioxide and hydrogen. The presence of both of these gases during the principal biological stage has been definitely established by Eyre and Nodder<sup>7</sup>, Ruschmann<sup>21</sup>, and others. To a large

extent these gases are prevented from escaping by the layer covering the surface of the retting liquor, and they may therefore accumulate in such quantities that the hydrogen can be burned with its characteristic bluish flame—a somewhat dangerous experiment—when allowed to escape through the film (Ruschmann<sup>21</sup>). The decomposition of the pectin by *Bac. amylobacter* also gives rise to the production of acids. The nature of these acids is indicated by the decomposition products formed by *Bac. amylobacter* from other carbohydrates, and they undoubtedly comprise acetic and butyric acids (Jackson<sup>22</sup> and Kayser and Delaval<sup>23</sup>). That substances such as acetone, ethyl alcohol, or butyl alcohol are also formed is probable from the investigations of Kayser and Delaval. Should it be shown by a careful analysis of the retting liquors that acetone and alcohol are absent, it must not therefore be concluded that they are not produced by *Bac. amylobacter*, since there are indications\* that acetone, at least, can be converted into other compounds by members of the *Bac. mesentericus* type. The amount of acid produced by the decomposition of the pectin will naturally depend on the concentration of this substance. It is generally considerably less than that formed from other carbohydrates during the preliminary biological phase.

With the more or less complete decomposition of the pectin by *Bac. amylobacter*, which at 20°C. may take about 100 hours, the production of acid ceases and the fibre bundles gradually become separated from the surrounding tissues. The retting now enters that part of the principal biological stage which Eyre and Nodder term the fourth stage, and which in many respects is the most important of all. It is during this phase that the fibre bundles become entirely separated and the retting thereby completed. The determination of the completion of the retting process is of the greatest importance from an industrial point of view. Where the fibre bundles are allowed to remain in the retting liquor for even a comparatively short time after the technical endpoint of the ret, the individual fibres of the bundles will begin to

\* Unpublished observations by the authors.

separate owing to the decomposition of the substances which bind them together. These substances are stated by Störmer<sup>2</sup> to consist of partly lignified pectin. They are less readily decomposed by micro-organisms than ordinary pectin. A prolonged exposure to the enzymes of retting liquors, however, will cause the lignified pectin to become dissolved and the retted bundles to be separated into their component fibres. This is termed 'over-retting'.

In view of the danger of over-retting it is of the greatest importance to be able to determine with some degree of certainty when the retting is sufficiently far advanced for the fibre bundles to be removed from the retting liquors. Practical experience has evolved several tests for this purpose, among them the 'loose core' test, which requires the woody core of the retted tissue to be completely separable from the fibres on gentle pulling (Davies<sup>24</sup>). What appears to be a far more reliable test has recently been proposed by Eyre and Nodder<sup>7</sup>. On the perfectly justifiable assumption that the production of acid during the retting process indicates an increase in the microbiological activity, and during the principal biological stage, therefore, a progressive liberation of the fibre bundles, Eyre and Nodder determine the rate of production of acid throughout the ret, and find that the rate of production of what they term *permanent acidity* (i.e. the acidity not due to carbon dioxide) varies characteristically with the stages of the retting. This is illustrated in the curve given in Fig. 9.

The anaerobic retting process, it will be seen, is divided by Eyre and Nodder into four stages, of which the first three coincide with the physical, and the preliminary and principal biological stages. The time during which the retted material may be safely left in contact with the retting liquor during the fourth stage may, it is claimed, be fixed with considerable certainty. It varies from a quarter to a third of the time which the retting process takes to pass through the two preceding stages.

The determination of the acidity during the retting may be done either by titration or by conductivity methods, and is in

both cases sufficiently characteristic for the circumscription of the four stages of the retting.

It is obvious that a method such as this must be of great importance for the study of such retting problems as still remain more or less obscure. Among these may be mentioned the question of the bearing of the ripeness of the fibre tissues on the retting process, the influence of chemicals on

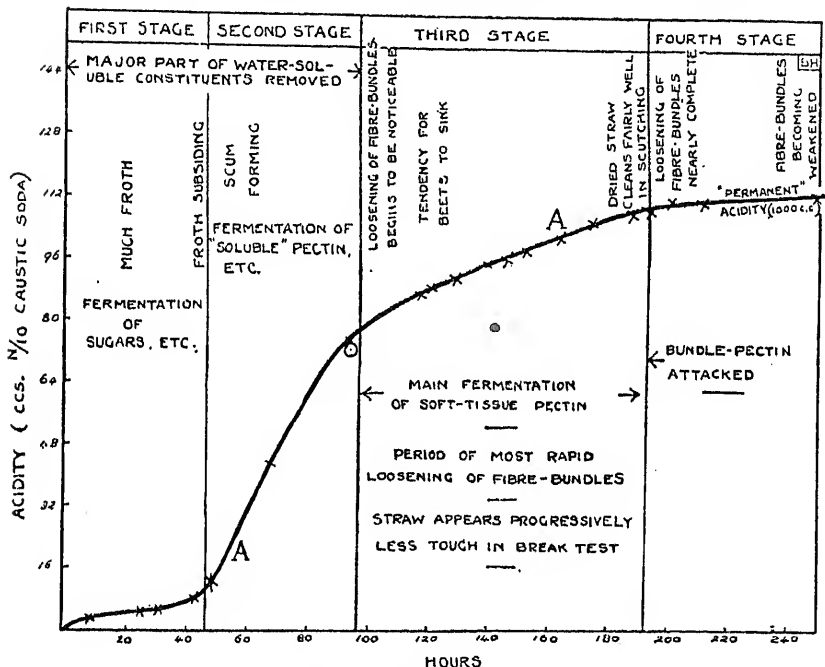


Fig. 9. The permanent acidity curve of a normal ret in stagnant water. (From Eyre and Nodder, in the *Journal of the Textile Institute*.)

the retting, and so on. The further investigations of these problems, foreshadowed by Eyre and Nodder, should be awaited with great interest.

It is during the principal biological stage, and particularly during the latter part of this stage, that the odours emanating from the anaerobic retting process are often so markedly offensive. The reason for their development is not fully understood, though it is assumed that they are due to



secondary microbiological decomposition processes involving a resolution of cellulosic materials. The odours are particularly noticeable in places where the retting is conducted in stagnant water, and where the debris of previous rettings has not been removed. In such cases Ruschmann<sup>21</sup> observed that methane was formed, a gas which is usually associated with the activity of anaerobic cellulose-decomposing bacteria.

After completion of the retting the tissues are removed from the retting liquor and dried either in the open or artificially, and either before or after a preliminary washing. The drying in the open is in some respects preferable to artificial drying, since the organic acids still adhering to the fibre bundles thereby become oxidized, probably through the action of aerobic micro-organisms (Ruschmann<sup>25</sup>). The subsequent steps in the mechanical stage consist in broad outline of the breaking and removal of the woody parts of the tissues and the combing and freeing of the fibre bundles from the adhering parts of the cortex.

It has already been mentioned that the anaerobic process may be carried out either in stagnant or in slowly-flowing water. The latter method is generally followed in Belgium for the retting of flax and is often carried out in two stages, particularly at Courtrai, on the river Lys. Here the retting is stopped during the principal biological phase, before the fibre bundles have been completely loosened. The flax straw is then removed from the river and stooked. After a time it is again placed in the river and allowed to stay there for about the same time as during the first part of the ret. The stooking is claimed to have a beneficial effect, probably of a biological nature. During the second stage of the retting the resolution of the remaining pectin proceeds very slowly and the danger of over-retting is in consequence greatly diminished. On the other hand the time taken to complete this type of retting process is seriously increased.

The question of time was of comparatively little importance in the days when the isolation of fibres was primarily a cottage industry, involving the treatment of small quantities of raw material only. With the development of industrial

plant designed to work throughout the year with large quantities of raw material and at a minimum cost, the speeding up of the anaerobic retting became a question of great importance. Nearly all of the many processes evolved during the last eighty years have, therefore, endeavoured to do this, and to do it by increasing the temperature at which retting is carried out. The original suggestion for speeding up the process by increasing the temperature dates from 1864 when Schenck recommended this method to the Irish retting concerns (see Hodges<sup>20</sup>). The rate at which the fibre bundles are liberated in the warm water rets is very appreciably increased, and under favourable conditions and at a temperature of about 37° C. the endpoint of the ret may be reached in from forty to fifty hours. The danger of over-retting is thereby seriously increased. For this reason many prefer to use a temperature of 28 to 29° C., even though the endpoint may not then be reached for 120 hours. From a microbiological point of view the principles of the warm water ret are identical with those of cold water retting. The mechanical and biological improvements which from time to time have been introduced into warm water retting are therefore equally applicable to cold water.

In their investigation of the anaerobic retting process Beijerinck and van Delden<sup>12</sup> established the two following important facts, that the changing of the water at the completion of the physical phase, and the supply of a limited amount of oxygen during the biological stages greatly favour the process. By the replacement of the liquor the substances extracted from the tissues are to a large extent removed and the food supplies of the secondary retting flora thereby curtailed. This again means a reduction in the amount of acids produced during the early part of the retting and the possibility of an increase in the ratio of tissue to water. Incidentally, colouring matter is also eliminated and the danger of a discoloration of the fibre bundles thereby diminished. By adopting Beijerinck and van Delden's recommendations, Herzog<sup>20</sup> succeeded in obtaining an excellent and practically odourless fibre containing as much as 89.6

per cent. of cellulose, as against 86.1 per cent. of cellulose in fibres prepared by the ordinary warm water process. Herzog replaced the water in his tank continuously, at a rate equal to four complete changes. Aeration was carried out to a moderate extent, warm air being blown through the liquor for five to ten minutes three times daily.

A further improvement in the anaerobic warm water ret has been claimed as a result of the addition of a pure culture of the specific retting organisms. For this purpose Störmer<sup>2</sup> used a strain of *Bac. amylobacter*, *Plectridium pectinovorum*, while Carbone<sup>27</sup> utilizes a special and little-known type, *Bac. felsineus*. Though many investigators have been unable to confirm Störmer's observations, it is generally conceded that the addition of a culture of *Bac. felsineus* is essential in the retting process devised by Carbone, which is at least equal to the best conducted warm water rets to which no addition of cultures of the anaerobic retting organisms is made.

*Bac. felsineus*, which was obtained by Carbone<sup>15</sup> from the mud of an Italian hemp retting pit, is a slender spore-forming rod measuring 0.3 to 0.5  $\mu$  by 3 to 5  $\mu$ . The spore measures 1.5  $\mu$  by 2.0  $\mu$  and is formed towards one end of the rod without causing a swelling of the cell. The natural habitat of *Bac. felsineus* appears to be hemp and the mud of hemp retting pits. Carbone has so far been unable to obtain it from any other source. In potato mash it develops well at 37 to 38° C., producing a considerable amount of frothing during the first twenty-four hours (Tobler<sup>28</sup>). At the same time the mash becomes bright orange in colour. The large particles of the mash show a tendency to rise to the surface of the liquid as they become permeated by the entrapped gas, and strands of mucilage may be observed extending from the surface of the mash towards the bottom of the container. The fermenting mash has a pleasant smell of bananas. As the culture becomes older its colour changes from a light to a darker brown. Calcium carbonate, if present in the mash, is gradually dissolved by the acids produced by the organism. In its stead the bottom of the container becomes covered with a snow-white layer of starch granules stated to have been set free

from the cells of the potato tissues. *Bac. felsineus* has not yet been obtained in strictly pure culture, since it does not develop on the laboratory media used for this purpose. It is not possible at the moment, therefore, to speak with certainty of its relationship to the *Bac. amylobacter* group (Ruschmann<sup>10</sup>).

As Carbone regarded his organism as an obligatory anaerobe, he originally recommended the addition of a saccharomycete, *Saccharomyces ellipsoideus*, to cultures of *Bac. felsineus*. Tobler<sup>28</sup> has shown, however, that this addition is unnecessary and that perfectly good growth can be obtained without it.

With a potato culture of *Bac. felsineus* as a starter and supplied in the proportion of 1 litre of starter to 10 kg. of dry tissues, a warm water ret of either hemp or flax can be completed in the Carbone process in from forty-eight to seventy-five hours, when conducted at 37 to 38° C. The odours of the liquor are not objectionable and the fibre bundles, when liberated, are much freer from adhering pieces of cortex than is the case in the usual warm water ret. The colour of the fibres is bright, and lighter than usual, and the yield is good. The liquor of the first ret may be used again to start a second, and this again for a third. After that, however, it is necessary to employ a fresh potato mash starter.

Interesting investigations on retting processes have also been carried out by Kraus<sup>18</sup>. This authority undertook to determine the value of two German patents, by Hosemann and Fiegel<sup>29</sup> and by Soltau<sup>30</sup>, who recommend the digestion at 37° C. of fibre tissue with pancreatic extracts in the presence of 0.5 per cent. of sodium bicarbonate. Kraus found that the methods yielded satisfactory fibres, but that equally satisfactory results could be obtained in the absence of the pancreatic extract, provided sodium bicarbonate was present in the retting liquor in a concentration of about 0.4 per cent. The method finally adopted by Kraus and Biltz, and embodied in a German patent (see Kraus<sup>31</sup>), is essentially a warm water ret in which the material is placed in a sufficient quantity of a liquid containing from 0.5 to 1.0 per cent. of sodium bicarbonate. Calcium bicarbonate may replace the more expensive sodium bicarbonate. A mixture of 0.05 per cent. of sodium

bicarbonate and 1 per cent. of calcium carbonate is also suitable. The endpoint of the ret is reached in two or three days, and the liquor is stated to remain neutral or slightly alkaline throughout the ret.

Another process carried out by anaerobic micro-organisms, which however must be classed among the cold water rets, is the Ochmann process, which has been favourably reported upon in Germany (Ruschmann<sup>32</sup>). The material to be retted is placed in concrete tanks and covered with water in the usual proportions. On alternate days this water is slowly run out through the bottom of the tank and is simultaneously replaced at the same rate by fresh water. The fresh water is run into the tank as a spray and thus becomes saturated with oxygen. By the repeated removal of the retting liquor, the harmful substances extracted from the fibre tissues and the decomposition products formed by the breakdown of sugar and pectin are constantly removed without disturbing the material undergoing retting. From the tank the spent liquor runs into a settling tank, in which heavier particles, such as soil and plant tissues, are deposited. By overflow the liquor leaves this tank and enters a second from below. This tank is filled with stones or similar material to assist the aeration of the liquor. The biological oxidation processes which the liquor thereby undergoes make it sufficiently pure to be used again. Any excess is disposed of to local farmers, who favour its use for watering grass land, probably on account of its content of phosphates and nitrates. Alternatively, it may be run off into streams, since it has been shown to be entirely harmless to fish. Trout have even been found collecting round the places where this liquor is emptied into the streams. As regards ease of disposal of the spent retting liquors, Ochmann's method would appear to be superior to all others, since the elimination of the liquors is often a serious problem on account of their acid content and their objectionable odours. A further advantage is the entire elimination of over-retting: the yield of long staple is in consequence very high, amounting to 80 per cent. of that theoretically possible. The total loss of fibre is stated to be only 6.5 per cent. (Ruschmann<sup>32</sup>).

Before leaving the subject of anaerobic retting it is necessary to mention the investigations carried out by Kayser and Delaval<sup>23</sup> to which reference has already been made in this and the third chapter. In the course of their investigations these authors isolated six different types of bacteria, which are stated to be capable of decomposing pectin and in consequence are able to ret hemp and flax. A short description of these organisms, of which five were favoured by the presence of oxygen and one was not, was given in Chapter III in discussing the pectin-decomposing bacteria. On testing the fermenting properties of these organisms it was found that all decomposed both monoses and disaccharides, the former better than the latter. Pectin was found to be more or less readily decomposed, the types No. 2 and No. 6 fermenting 56.7 per cent. and 48 per cent. respectively of the pentoses present in the pectin. From a study of the liquors collected from rets carried out with the six strains, Kayser and Delaval arrived at the conclusion that the chemical changes taking place during retting vary not only with the type of micro-organism used, but also with the material retted, the temperature, the nature of the water, and the method of retting.

It is readily understood that the material retted, the method of retting, and the nature of the water can influence the nature of the decomposition products, the last named through neutralization of the acids formed. It is more difficult to see how a change in the temperature can do so, unless it is assumed that certain species of micro-organisms are suppressed by a rise or fall in temperature and others, which produce different enzymes, are favoured under such conditions.

Of decomposition products of an acidic nature the following were identified by Kayser and Delaval in the various retting liquors: formic acid, acetic acid, butyric acid, succinic acid, and lactic acid. In addition, ethyl alcohol, acetone, sugars, hydrogen, carbon dioxide, and soluble nitrogen compounds were obtained. As in other anaerobic retting processes Kayser and Delaval found it beneficial to renew the liquors in their experimental rettings after the completion of the physical

phase, and they remark that in dealing with some fibre plants, as for instance ramie, it is absolutely essential to do this.

In order to study the action of each of their six strains of bacteria on the fibre tissues it is clear that Kayser and Delaval had to work with tissues previously sterilized. The sterilization of fibre plants without doing damage to the pectin or other constituents of the tissues has always been regarded as a difficult matter, which frequently led to misleading conclusions when carried out without sufficient precautions. Heating of the tissues with steam was resorted to by Hauman<sup>10</sup>, but this cannot be regarded as satisfactory, since drastic heating affects the pectin, while moderate heating is often insufficient to destroy the spores of some of the bacteria adhering to the tissues. Sterilization by means of antiseptics, therefore, has been frequently employed. For this purpose Kayser and Delaval used carbon bisulphide, sodium fluoride, or *eau de Javel*. A better method would appear to be that recommended by Kraus<sup>11</sup>, who places the tissues for several days in a closed container in which sufficient chloroform is present to saturate the tissues with the vapour. After sterilization all traces of chloroform are removed from the tissues by aeration of the container with sterile air.

The investigations of Kayser and Delaval have led to the taking out of a patent by Kayser<sup>13</sup> for the retting of textile plants by the above-mentioned strain No. 6.

**Aerobic retting processes.** Reference has been made in the preceding pages to the observation that a restricted aeration of the retting liquors is beneficial even in anaerobic retting processes. Beijerinck and van Delden<sup>12</sup> went so far as to suggest that a limited supply of oxygen favoured the development of *Bac. amylobacter* or, as they termed their strain of this type, *Granulobacter pectinovorum*. Recent experience with the so-called Rossi retting process<sup>14</sup> makes it more probable, however, that the beneficial action is an indirect one, due to the development of micro-organisms which break down the acid formed by the anaerobic forms. In any case, Ruschmann<sup>25</sup> has shown that in the retting of flax and hemp the production of acid is inversely proportional to the degree

of aeration of the liquor and that *Bac. amylobacter* may be almost completely suppressed in vigorously aerated liquors and be superseded by aerobic forms. Processes in which the suppression of *Bac. amylobacter* is aimed at may be termed *aerobic* water retting processes. That aerobic bacteria can be used for retting purposes was pointed out by Hauman<sup>10</sup> in the early days of the study of these processes, and these observations have been confirmed by other investigators, notably by Behrens<sup>11</sup> and by Beijerinck and van Delden<sup>12</sup>. Not until comparatively recently, however, have such bacteria been utilized industrially for retting processes, as in the Rossi process<sup>34</sup>. Here a current of air is forced through the liquor, which is maintained at a temperature of 28 to 30° C., after previous inoculation with a starter, a culture of *Bac. comesii*, of which a short description was given in Chapter III. The rate at which air is forced through the liquor is about 200 litres per minute in a tank of 50,000 litres capacity (Carter<sup>35</sup>). At this rate, however, *Bac. amylobacter* and other obligatory and facultative anaerobes are not entirely suppressed and the retting liquors are therefore slightly acid. The partial development of the anaerobic forms is of advantage according to Ruschmann<sup>36</sup>, since they suppress the formation of slimy growths of fungi and aerobic bacteria on the fibre tissues, which occur when the liquor is too vigorously aerated. When properly conducted the Rossi process is completed in about two days when hemp or flax is retted. The danger of over-retting is claimed to be entirely eliminated, if the rate of aeration is somewhat increased towards the end of the retting. This, however, does not appear to be substantiated by actual facts, and can hardly be so in view of the wide distribution of aerobic cellulose-decomposing bacteria in nature. In the writers' experience, destruction of fibres retted by aerobic processes occurs through the action of such bacteria, when the retting process is allowed to continue for three to four days after the endpoint of the ret. An advantage of the Rossi process is that the liquors may be used as starters for fresh rets for a considerable number of times. The replacement of the liquor after the physical phase is not necessary,



and may in fact be undesirable (Ruschmann<sup>36</sup>), since the substances extracted from the tissues serve as food material for *Bac. comesii*. As the spent liquors are almost inodorous and contain little acid, their disposal offers no serious difficulties.

Besides *Bac. comesii*, a number of other aerobic bacteria undoubtedly exists which might carry through the aerobic water retting processes. Ruschmann states that he has isolated two such types.

In a review of the respective merits of the various warm water retting processes discussed in this chapter, Ruschmann<sup>37</sup> records some interesting data. While a sample of flax retted by the ordinary warm water ret yielded 17 per cent. of fibre, the same flax gave 16.1 per cent. under the Carbone process and 16.9 per cent. under the Rossi process. The tensile strength of the fibres was found to be greatest in the sample retted by Rossi's process and lowest when prepared by Carbone's process. The retting was generally completed sooner in the Rossi process than in the other two, and last in the ordinary warm water process. These figures, though interesting, should not be regarded as a final judgement of the merits of the three methods. Thus, Carbone<sup>38</sup> criticizes the results obtained by Ruschmann with the Carbone process, on the grounds that an inferior flax-straw was used and that too high a temperature (70 to 80° C.) was applied in drying. Considerably more evidence is undoubtedly required before a final decision can be given as to the best method for conducting the warm water retting process.

Aerobic retting may be conducted in an entirely different way to that already described, and has in fact been thus carried on for centuries, particularly in Russia, by a method termed *dew-retting*. The fibre plants are spread in thin layers on the ground during summer, early spring, or winter. The ground selected is preferably heathland or any other poor land containing few micro-organisms. Direct contact of the material to be retted with the soil is avoided as far as possible. Tobler<sup>39</sup> recommends placing the fibre tissues on a surface composed of such typical heath and moor vegetation as *Deschampsia flexuosa*, *Nardus stricta*, *Bromus sterilis*, *Calluna*

*vulgaris*, *Epilobium angustifolium*, *Hypericum perforatum*, *Rumex acetosella*, and others, which are particularly suitable for the purpose since they are rigid enough to form a firm and porous substratum for the fibre tissues. When spread, dew and rain keep the fibre plants sufficiently damp to enable micro-organisms to develop. The microflora appearing on the tissues has been investigated from time to time, and though most opinions agree that fungi are chiefly responsible for the liberation of the fibre bundles, many still differ as to the actual species of fungi which must be regarded as the causative agent. In his examination of a sample of flax prepared by the dew-retting method, Hauman<sup>10</sup> isolated the following types of micro-organisms: *Cladosporium herbarum*, *Bact. coli commune*, *Bac. mesentericus*, *Bac. subtilis*, *Bac. mycoides*, *Bac. termo*, *Bact. fluorescens liquefaciens*, *Micrococcus roseus*, *Penicillium glaucum*, and *Mucor mucedo*. Of these Hauman regards *Cladosporium herbarum* as the chief retting organism.

Behrens<sup>40</sup> in his investigation of the dew-retting of hemp arrived at the conclusion that *Cladosporium herbarum* was not the causative agent, but was to be regarded as a dangerous infection, responsible for the black discoloration of the finished fibres. In its stead he placed two species of *Mucorineae*, *Rhizopus nigricans*, also called *Mucor stolonifer*, and *Mucor hiemalis*. The former is stated to be the retting organism of dew-retted fibres prepared during the summer and autumn months, and the latter the retting organism of winter dew-retted fibres. Neither of these fungi is capable of attacking cellulose, in which respect they differ from *Cladosporium herbarum*, *Botrytis cinerea*, and *Aspergillus* species, which may also be met with on dew-retted fibres. Beijerinck and van Delden<sup>12</sup> also regard dew-retting as caused by fungi, but do not indicate the species. Rusehmann<sup>41</sup> has more recently subjected two types of hemp and three types of flax to dew-retting with a view to studying the microflora of this process. He states that *Cladosporium herbarum* was undoubtedly the chief, if not the sole, retting agent in his experiments. In the summer-retted samples he also observed the following forms: *Mucor plumbeus*, *Aspergillus* species, *Penicillium* species,

yeasts, *Oidium* species, *Bact. coli* types, *Cocci*, *Bac. Megatherium*, and *Bac. mesentericus*. On the hemp samples *Rhizopus nigricans* was also met with. All of these species, however, disappeared from the tissues as *Cladosporium herbarum* developed, and before the parenchymatous tissues showed signs of becoming loosened. The mycelium of the *Cladosporium* finally covered the whole of the tissues, except in places where bacteria, *Penicillium*, *Oidium*, and yeast species had accumulated. These places were often but sparsely overgrown with *Cladosporium* and sometimes not at all. The thick layer of *Cladosporium* growth adhered firmly to the surface of the material undergoing retting, as a dark green or almost black covering. The mycelium was also found extensively distributed between the cells of the cambium, the phloem, and the cortex. In the winter-retted samples the development of *Cladosporium* was less uniform and the retting in consequence more irregular. Neither *Rhizopus nigricans* nor *Mucor hiemalis* was found. Ruschmann found the dew-retting completed in seven days during summer, and in about fourteen days during winter. The winter samples, however, were unevenly retted, even after a fortnight, presumably on account of the uneven development of the *Cladosporium*.

As a result of his investigations Ruschmann concludes that the two *Mucor* species of Behrens take little or no part in dew-retting, that *Mucor plumbeus* may be able to ret, but, like *Rhizopus nigricans*, is unable to compete with *Cladosporium herbarum*. He agrees that aerobic bacteria may be able to carry out the dew-retting in the absence of *Cladosporium*, but that anaerobic forms such as *Bac. amylobacter* take no part in the process. In this respect he differs from Störmer, quoted in Lafar's text-book on mycology<sup>42</sup>, and thus confirms Steglich's observations. The fact that the spores of *Cladosporium herbarum* are always found on dew-retted fibres is regarded by Ruschmann as proof of the importance of this fungus.

Interesting though Ruschmann's investigations undoubtedly are, they hardly decide the question of the nature of the microflora responsible for the dew-retting of fibre plants.

This question must therefore remain open until such time as researches on a large number of fibre plants can be carried out, preferably at places where the process is used technically.

The above outline of retting processes, viewed primarily from the standpoint of flax and hemp, applies to other textile plants such as *Corchorus* (jute), *Boehmeria* (ramie), and *Urtica* (nettle). In these cases, however, retting is not usually conducted as carefully as in the case of flax and hemp. Most primitive would appear to be the methods by which coir is separated from the coco-nut husk. According to Fowler and Marsden<sup>43</sup> the retting of these husks in sea or brackish water requires ten to twelve months for completion, except where special precautions are taken to renew the retting water. In this case the reaction may last only three months. Such prolonged exposure to a variety of different micro-organisms, including many cellulose-destroying forms, could not be contemplated except in the case of such heavily lignified fibres as coir.

The fact that the isolation of vegetable fibres is largely carried out by microbiological means, and under conditions which to some extent favour the development of cellulose-destroying micro-organisms, cannot but have an effect on the subsequent fate of such fibres. This interesting question will be further dealt with in Chapter XI.

**Preparation of starch by the fermentation of pectin.** The first method for the isolation of starch by a microbiological process dates from 1839, a period when the nature of micro-organisms was as yet hardly realized. It was evolved by Völker<sup>44</sup>, who was granted a patent for the preparation of potato starch by fermentation. In his process raw potatoes are pressed in order to remove as much of their moisture content as possible. The remaining pulp, still containing about 50 per cent. of moisture, is piled in heaps, with alternate layers of a porous material, such as twigs, to ensure aeration. Spontaneous heating soon raises the temperature sufficiently for the desired fermentation to begin. During the fermentation the pressed potato mass is reduced to a fine pulp from which the starch may be isolated by mechanical means such as washing through a sieve.

An insight into the changes which the potato tissues undergo during Völker's process may be obtained by a repetition of Mitscherlich's<sup>5</sup> method for the preparation of a so-called 'cellulose-dissolving ferment' from potatoes. Mitscherlich, it will be recollected, steeped slices of raw potato in luke-warm water and left them to decay. He observed that a brisk fermentation started in the maceration when kept at a temperature of about 30° C., and that the potatoes gradually disintegrated to a whitish pulp collecting at the bottom of the container. Mitscherlich was of the opinion that the cellulose of the cell walls of the potato tissues had been resolved during this fermentation and that the starch had in consequence been liberated. That, however, is not the case. What occurs is that the middle lamellae of the potato tissues become resolved, and the liberated cells, with their starch content more or less intact, are deposited as a precipitate. The changes to which potatoes are subject both in the Völker process and the Mitscherlich fermentation are therefore those of a typical pectin fermentation and are undoubtedly caused by members of the *Bac. amylobacter* group.

The preparation of wheat starch by a similar fermentation has also been commercially exploited (Fesca<sup>45</sup>). In this process the grain is first softened in water, crushed between rollers and then left to ferment, preferably after inoculation with material from a previous fermentation. The fermentation, which is stated to produce acetic and lactic acids, is a complicated reaction involving decomposition of both pectin and starch. It is completed in from 8 to 20 days, by which time the liquid standing over the crushed grain has become clear. The starch contained in the liberated endosperm cells is isolated by mechanical means.

Neither this nor the Völker process appears to have gained a firm footing commercially. In his account of the preparation of potato starch, Saare<sup>46</sup> ascribes this to the fact that the starch is in some way altered during the pectin fermentation. It is found to precipitate much less readily from its suspensions than mechanically prepared starch. The fermentation process

nevertheless possesses an important advantage inasmuch as the starch can be extracted more thoroughly from the tissues than is possible in the purely mechanical process. Saare estimates that the latter processes are responsible for a loss of 15 per cent. of the total starch available, and he suggests that this might be recovered by subjecting the residues to a fermentation process. In trials he found that about 40 per cent. of this starch could thus be recovered. The starch, however, was of the same inferior quality as other 'retted' starches, and settled badly from suspensions. A careful study of these fermentation processes is almost certain, as Saare suggests, to result in improvements, and there is little doubt that better types of micro-organisms could be found for this purpose than *Bac. amylobacter*, which possesses diastatic enzymes and therefore shows a tendency to attack the starch itself. Tobler's<sup>28</sup> recent observations on the growth of *Bac. felsineus* in potato mashes indicate that this organism might be more suitable.

The microbiological decomposition of pectins is of primary importance in some other directions, for instance in the 'wet rot' of clamped potatoes and in the rotting of stored fruits. These decomposition processes will not be dealt with here; a description of them may be found in most text-books on plant diseases. Readers particularly interested in their study are referred to the investigations of Behrens<sup>47</sup>, Kramer<sup>48</sup>, Wehner<sup>49</sup>, Jensen<sup>50</sup>, and Appel<sup>51</sup>.

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## CHAPTER VIII

### HEMICELLULOSES AND CELLULOSE

THE presence of hemicelluloses in appreciable quantities in all plants makes their natural decay, their microbiological decomposition, a question of considerable interest. Though this decomposition occurs in the soil, in the manure heap, in the intestine, in the sea, and in fresh water, its importance can hardly have been fully appreciated, judging by the scanty and scattered information available.

It was mentioned in the introductory chapter that the hemicelluloses may be divided into two natural groups, one comprising the types which act as reserve food materials for the plants which produce them, and the other serving as additional structural support for the cellulose skeleton of vegetable tissues. The microbiology of the reserve hemicelluloses is of interest mainly from the point of view of their intestinal decomposition. Their destruction appears to proceed on the lines of that of the structural hemicelluloses (Hérissey<sup>1</sup>, Rippel<sup>2</sup>, Pringsheim<sup>3</sup>), and will not be dealt with separately. Their proposed utilization in industry for the production of ethyl alcohol will be discussed in Chapter XIII.

Since hemicelluloses are insoluble in water and at the best only form colloidal suspensions with this solvent, the first stage in their microbiological destruction must be their conversion into soluble compounds. This stage was studied by Hérissey<sup>1</sup>, Schellenberg<sup>4</sup>, and Schmidt, Peterson, and Fred<sup>5</sup> in the case of certain hemicellulose-decomposing fungi, and by Gran<sup>6</sup> and Sawamura<sup>7</sup> in the case of hemicellulose-fermenting bacteria. Through this resolution the hemicelluloses are converted into monoses, either hexoses, such as galactose and mannose, or pentoses, such as arabinose and xylose.

The second stage involves a breakdown of the monoses



produced during the first stage. Where the monoses formed are hexoses, their further decomposition can be readily contemplated. It may be performed either by the original microflora or by a variety of secondary micro-organisms, incapable of attacking the insoluble hemicelluloses. The decomposition products of this stage, therefore, depend entirely on the micro-organisms present and may range from organic acids, such as acetic, butyric, and lactic acids, to ethyl alcohol, and finally carbon dioxide and water. In the first case, types of the *Bac. amylobacter* group, the *Bac. mesentericus* group, or the *Bact. coli* group may be active; in the second, yeasts or certain alcohol-producing bacteria; and in the third, probably mainly hyphomycetes. The hexoses may serve also as a carbohydrate supply for denitrifying and nitrogen-fixing micro-organisms and may thus assist in the liberation and fixation of this important element.

Where the hemicelluloses yield pentoses on resolution (hydrolysis), the secondary microflora participating in the destruction of the resulting monoses is less varied. It may still include members of the *Bac. amylobacter*, the *Bact. coli*, and particularly the *Bact. lactipentouacetium* groups, as well as a variety of hyphomycetes and many ammonia-producing bacteria, such as *Bac. Megatherium*. Some pentosans, particularly xylan, are readily decomposed by the latter types (Stoklasa<sup>8</sup>). Nevertheless, the wide distribution of pentosans and pentoses, even in arable soils (Shorey and Lathrop<sup>9</sup>), is an indication that pentoses are not as readily decomposed by micro-organisms as hexoses, which are not found in arable soil in measurable quantities.

In many ways fungi appear to be specially adapted for the decomposition of hemicelluloses. Reference to such fungi was made in Chapter V. In Chapter III an account was also given of the few bacteria which have been found capable of decomposing these polysaccharides. How far actinomycetes decompose hemicelluloses is not yet quite clear. Henneberg's<sup>10</sup> investigations of the intestinal microflora point to their importance in this respect, but more definite information is undoubtedly required.

The microbiological decomposition of *cellulose* proceeds, like that of the hemicelluloses, in two stages, the first involving an hydrolysis of the cellulose resulting in the formation of the soluble carbohydrates cellobiose and glucose, and the second the further oxidation of these sugars either by the cellulose decomposers themselves or by a secondary microflora. In the laboratory this decomposition process is never carried through to completion. There will always be left over a more or less bulky residue, which may be coloured ochre, blackish, or even reddish. Examined microscopically, this residue shows the structure of the original cellulose material. Thus where filter paper is being decomposed, the original fibrous structure can be readily discerned. The extent to which the residue is changed chemically during attack by micro-organisms has not been determined. When boiled in a dilute solution of alkali, part of the residue is dissolved. There are no indications, however, that this soluble part is in any way related to humic substances.

The ochre pigment formed by many cellulose-decomposing bacteria was examined by Hutchinson and Clayton<sup>11</sup> in their investigations on *Spirochaeta cytophaga*. They found that the pigment is soluble in the ordinary fat solvents, yielding a canary-coloured solution with ether, an ochre-coloured solution with petroleum ether or chloroform, and an orange-coloured solution with carbon bisulphide. On evaporation of these solutions an oily orange-coloured residue remained. The pigment gives colour reactions resembling those of carotin. It therefore resembles the lipochrome pigments found in many bacteria, for instance in *Staphylococcus pyogenes aureus*.

The formation of copper-reducing carbohydrates as intermediate decomposition products during the microbiological decomposition of cellulose was foreshadowed by von Euler's<sup>12</sup> experiments on the action of a juice pressed from *Merulius lacrymans* on cellulose dextrins. The formation of cellobiose as a result of the microbiological decomposition of cellulose was demonstrated by Pringsheim. In his treatise on the polysaccharides, Pringsheim<sup>13</sup> gives a lucid account of his investigations in this direction. By the addition of antiseptics, particularly a 0.5 per cent. solution of iodoform in acetone, he succeeded in

arresting the growth of cellulose-decomposing bacteria without materially affecting the activity of their enzymes. In this way he prevented the absorption and further oxidation by living cells of the soluble intermediate decomposition products of the cellulose. The cessation of growth, without a destruction of the hydrolysing enzyme, was also attained by raising the temperature of a vigorously fermenting culture of thermophilic cellulose-decomposing bacteria to a point above the maximum temperature for growth, but below the thermal point of destruction of the hydrolysing enzymes. In both cases Pringsheim observed the accumulation in his cultures of copper-reducing carbohydrates, which he found to consist of cellobiose and glucose. It is interesting to note that Pringsheim found two enzymes necessary for the conversion of cellulose to glucose, one termed cellulase, hydrolysing the cellulose to cellobiose, and another, cellobiase, converting the cellobiose to glucose. As the thermal point of destruction of the cellobiase was found to be lower ( $67^{\circ}\text{C.}$ ) than that of the cellulase ( $70^{\circ}\text{C.}$ ), it was possible to prevent the formation of glucose and to limit the hydrolysis of the cellulose to cellobiose by conducting the fermentation of the cellulose at a temperature between  $68$  and  $69^{\circ}\text{C.}$  A further method for the accumulation of cellobiose is suggested by Groeneweg<sup>14</sup>, who states that this disaccharide is produced more rapidly than it is decomposed when the cellulose fermentation is conducted under slightly alkaline conditions.

Under ordinary conditions neither glucose nor cellobiose accumulates to any measurable extent. This is important, since cellulose-decomposing micro-organisms may be inhibited in their growth by the presence of even small quantities of copper-reducing carbohydrates (Hutchinson and Clayton<sup>11</sup>). These sugars are nevertheless present in sufficient quantities to serve as a source of energy for other micro-organisms. Thus Pringsheim<sup>15</sup> found that a culture of Onelianski's *Bac. methanigenes* growing on cellulose assimilated nitrogen from the air when inoculated with a nitrogen-fixing strain of the *Bac. amylobacter* type. Through the oxidation of the cellobiose and glucose by *Bac. amylobacter* the culture accumulated

10.4 mgs. of atmospheric nitrogen per gramme of cellulose decomposed, or 10 kgs. of nitrogen per metric ton of cellulose fermented. This observation serves as a striking example of the importance of those microbiological processes by which cellulose is destroyed in nature, particularly in the soil. It also emphasizes the importance of the secondary microflora associated with the true cellulose decomposers in the natural decay of cellulose.

In the laboratory the presence of the secondary microflora in crude cultures of cellulose-fermenting bacteria is a constant source of anxiety, making the preparation of pure cultures of the true cellulose decomposers an almost impossible task. This difficulty, however, does not necessarily justify the assumption, so often advanced in the early days of the investigation of the microbiological decomposition of cellulose, that many cellulose-destroying bacteria cannot be artificially cultivated under any conditions except in symbiosis with other types.

Though cellobiose and glucose may act as inhibitory substances in preventing the development of cellulose-decomposing bacteria, it is nevertheless through the oxidation of these carbohydrates that all true cellulose fermenters acquire the energy needed to perform their various life functions. The final decomposition products which may result from this oxidation of cellobiose and glucose were mentioned in Chapter III in describing Omelianski's and Khouvine's anaerobic cellulose decomposers. These products comprise carbon dioxide, hydrogen, methane, organic acids, such as butyric and acetic acid, and ethyl alcohol. The last-named substance is usually found only in very small quantities. In two cases, in the decomposition of cellulose by *Bac. cellulosaе dissolvens*, Khouvine<sup>16</sup>, and by Fred, Peterson, and Viljoen's thermophilic cellulose decomposer<sup>17</sup>, yields are reported to be higher, amounting to 5 to 25 per cent., calculated on the cellulose fermented. The formation of this alcohol, as well as of the methane and ethane observed, is ascribed by van Senus<sup>18</sup> to the reducing action exercised by the evolved hydrogen on the primary cellulose-decomposition product, the acetic acid.

Another attempt to study the chemical reactions involved

in the formation of fermentation products of cellulose was made by Neuberg and Cohn<sup>19</sup>, who found that acetaldehyde could be isolated from cultures in which cellulose was being fermented. It is noteworthy that Neuberg and Cohn utilized impure cultures of methane- and hydrogen-forming bacteria for their experiments and that these cultures also gave acetaldehyde when grown in glucose medium in which *Bac. methanigenes* and *Bac. fossilicularum*, Omelianski<sup>20</sup>, do not develop. For this reason their experiments cannot be regarded as conclusive since the production of the acetaldehyde may have been the result of the activity of glucose-fermenting secondary micro-organisms present in their cultures.

Much work is needed before the biochemical reactions involved in the breakdown of cellulose are understood and the fermentation of cellulose, and of its associated substances, can be expressed by chemical formulae. At present such formulae would be of little, if any, value. The researches of the immediate future must concentrate on the acquisition and tabulation of facts, and first and foremost on the isolation and cultivation of the responsible micro-organisms in pure culture.

It is with a view to assisting in the tabulation of already acquired facts, and to rendering the subject less unwieldy, that the account of the natural decomposition of hemicelluloses and cellulose now to be given has been divided under four headings; their decomposition (1) in the manure heap, (2) on and in the soil, (3) under water, and (4) in the intestine. When the chemical reactions of this decomposition and the microflora concerned have been more thoroughly elucidated, a better grouping will no doubt suggest itself. At the moment that proposed offers distinct advantages.

Up to the present no account has been given of the microbiological decomposition of lignin and of 'suberin' (Chevreul<sup>21</sup>), both substances of the greatest importance. The reason for this apparent oversight is that nothing very definite is known, except that both lignin and suberin are more resistant to microbiological destruction than other vegetable substances. It must be admitted, however, that the eumycetes have frequently (<sup>22, 23</sup>) been reported as capable of decomposing lignin,

and it is a well-known fact (von Tubeuf<sup>24</sup>) that many wood-destroying fungi can be recognized by the appearance of the wood attacked by them, since some forms leave patches of pure cellulose in the decayed wood, from which hemicelluloses and lignin have been removed. The extent to which lignin is removed from wood during its destruction by fungi was estimated by Johnsen and Lec<sup>22</sup>, who found 30 per cent. of lignin destroyed by *Trametes pini* as against 15 per cent. of cellulose. In other investigations Bray and Andrews<sup>25</sup> and Johnsen and Hovey<sup>26</sup> report that the lignin remains undecomposed during the destruction of wood by fungi. This divergence of view is undoubtedly attributable to the fact that lignin is decomposed by some wood-destroying fungi and not by others. It is hardly justifiable therefore, as Fischer<sup>27</sup> has done, to quote the work of Bray and Andrews in support of his and Schrader's recently advanced theory on the formation of coal, without taking into account the many equally well-founded observations by other workers, that lignin can be decomposed by eumycetes. It will be necessary to return to this subject later, when discussing the microbiological aspect of coal formation.

An attempt to determine whether lignin can be fermented by bacteria was recently made by Pringsheim and Fuchs<sup>28</sup>. This, so far, is the only account of the decomposition of lignin by bacteria apart from some experimentally unsupported statements by Hébert<sup>29</sup> in 1892. For their purpose Pringsheim and Fuchs subjected wood to a boil with alkali, precipitating with acid the material thus extracted and converting it into the corresponding ammonium compound by neutralization with ammonia. This material still contained 6 per cent. of pentosans.

The ammonium compound was introduced into a flask containing 0.4 per cent. ammonium sulphate, 0.1 per cent. di-potassium hydrogen phosphate, and 0.5 per cent. crystalline magnesium sulphate, all of the salts being dissolved in tap water in which a few grammes of chalk were suspended. The flask was inoculated with soil and incubated at 37° C.

A slight gas evolution was visible in the flask after a few days. In sub-cultures of the same medium, however, no gas

was formed. The third sub-culture was examined after eight days' incubation to determine the amount of lignin fermented. It was found that out of the 10 grammes of lignin compound originally present, 6.5 grammes could be recovered after fermentation. Where the lignin was fermented in a concentration of 1 per mille, instead of 2 per mille as in the above experiment, only 4 grammes of lignin were recoverable from 10 grammes taken. In control experiments, where the lignin compound was introduced into the above medium and incubated at 37° C. for eight days, 4.5 grammes could be recovered from 5 grammes taken. Part of the lignin compound used had therefore undergone changes during fermentation which prevented its recovery. The nature of these changes was not studied by Pringsheim and Fuchs: nor has the result been given of the investigation of the responsible microflora, a study undertaken in collaboration with Liechtenstein. Both of these questions are subjects of great importance and, when elucidated, may go far to explain many points of interest, particularly to agriculture. As an example, it may be mentioned that Gray and Chalmers<sup>30</sup> found that traces of lignin act as an accelerator in the fermentation of cellulose by *Microspira agar-liquefaciens*.

The subject of the microbiological decomposition of suberin is entirely unexplored, though it would undoubtedly be a matter of great interest to establish the extent to which the vast amount of this substance accumulating yearly takes part in the production of humus, peat, and coal. It is perhaps an indication that the resistance of suberin is less marked than is generally assumed, that Fleming and Thaysen<sup>31</sup> found that cotton hairs which had been damaged by micro-organisms behaved differently to normal hairs when subjected to swelling treatment with carbon bisulphide and alkali. The cuticle of the damaged hairs had lost its property of resisting the expansion of the swelling cellulose, thus giving to the damaged hairs an abnormal microscopical appearance (see Figs. 12 and 13 in Chapter XI).

The natural decay of hemicelluloses and cellulose, including lignocellulose and suberin, was classed above under four headings;

the destruction (1) in the manure heap, (2) in the soil, (3) under water, and (4) in the intestine. In all four cases the destruction goes hand in hand with a microbiological change of other constituents of the plant materials involved, primarily with the destruction of the gums, the pectin, the nitrogen compounds, and the inorganic salts. Where information is available on the fate of the gums and the pectin, special reference will be made to these substances in the following pages. The nitrogen compounds and the inorganic salts will not be dealt with except in so far as they may have a direct bearing on the microbiological changes suffered by the cellulose and its associated substances.

(1) **The microbiological decomposition of hemicelluloses and cellulose in the manure heap.** Both in the manure heap, and in the compost heap the microbiological changes, resulting in the 'ripening' of the heap, aim at the conversion of the vegetable tissues present into compounds which will assist in maintaining and increasing the fertility of the soil. Primarily the heap is converted into the so-called *beurre noir* of the early French investigators, now generally described under the name of humic substances. To carry out these changes in the soil itself has long been known to be deleterious to plant life unless sufficient time is allowed to elapse for a fresh state of biological equilibrium to be established. This harmful effect was demonstrated by Fred<sup>32</sup> in the case of green manure. Where seed was sown immediately after ploughing in green plants as a manure, the seeds, or the seedlings germinating from them, were destroyed by fungi, and possibly bacteria, active in the conversion of the green manure. The micro-organisms thus attacked both the tissues which they were intended to destroy, and the other forms of vegetation present within the sphere of their activity. Not until the equilibrium of the soil-flora has been restored, on the complete destruction of the green manure, can plant life benefit from the resulting decomposition products. In the case of green manure this destruction is usually completed in a few weeks.

Another reason for the unfavourable action on the immediate development of plant life resulting from the introduction of



undecomposed plant material into the soil is claimed by Viljoen and Fred<sup>33</sup> to be the reduction of the nitrogen content of the soil due to the destruction of the tissues by cellulose-decomposing micro-organisms. By conducting the rotting of vegetable tissues on the manure heap, instead of in the soil, these harmful consequences are entirely overcome. In the heap the course of the conversion of plant tissues into manure will depend not only on the type of vegetable matter present, but equally on some of the materials of which the heap is composed, primarily on the form and the quantity of the nitrogen available. According to the nature of the nitrogen present, the manure heap may be classed as farmyard manure or compost manure. Farmyard manure consists essentially of the solid and liquid excreta of animals of the farmstead, mixed with varying quantities of straw and other binding materials. Compost manure is built up exclusively of vegetable debris, possibly mixed with sand or soil, but with no nitrogen beyond the supplies originating from the plant tissues.

As the changes suffered by the plant material are essentially the same in both the farmyard manure heap and the compost heap, the two rotting processes will not be discussed separately. It should be remembered, however, that the active microflora probably differs considerably in the two cases. Miehé's<sup>34</sup> investigations of the heating of accumulated vegetable matter indicate that cunycetes play a greater part in the rotting when air can gain access to the plant tissues, as in the compost heap, than when oxygen is more or less absent, as in the farmyard heap.

The processes involved in the ripening of farmyard manure were early subjected to careful investigation, and the volume of available literature testifies to the importance attached to the subject. One of the most recent contributions is of particular interest and will therefore be considered first.

In their investigation on the preparation of artificial farmyard manure Hutchinson and Richards<sup>35</sup> found that straw could be converted into a *beurre noir*, a brown plastic compound typical of a well-rotted farmyard manure, when

inoculated with certain micro-organisms, among them *Spirochaeta cytophaga*. Further experiments revealed that it was the food materials added to the straw with the culture of micro-organisms, rather than the latter, which facilitated the conversion. This observation did not indicate, of course, that the presence of micro-organisms was immaterial, but merely showed that the microflora normally present on the straw was capable of performing the decay under favourable conditions, that is, in the presence of food materials and moisture. The essential food materials were found to be nitrogen compounds, preferably urea. Cyanamide or ammonium salts could replace urea, if necessary. The rotting of the straw was favoured also by the addition of lime or chalk, probably on account of their neutralizing action, and by conducting the process at a temperature somewhat above that of the surrounding atmosphere. This latter requirement did not have to be artificially established, since the presence of oxygen in and around the rotting straw sufficed to raise its temperature by 15 to 20° C., once moisture and a source of nitrogen had been supplied. The importance of oxygen in this connexion was already known and had been observed by the earliest investigators studying the ripening of the manure heap, notably by Dehérain<sup>36</sup> and Dupont<sup>37</sup>.

The rise of temperature was ascribed by Dehérain to oxidation processes, initiated partly by the activity of aerobic micro-organisms, but principally by purely chemical processes. Dupont's, as well as Mische's<sup>34</sup>, investigations on the spontaneous combustion of hay have made it practically certain, however, that the rise of temperature is due entirely to microbiological oxidation processes. Thus Mische showed that samples of sterilized hay gave no rise of temperature until inoculated with suitable organisms.

Though Hutchinson and Richards found the presence of nitrogen essential for the conversion of straw into manure, they emphasize that the addition of an excess of nitrogen may retard, and in extreme cases may even prevent, the conversion. They found it important that the nitrogen should not exceed 2 per cent. of the weight of the dry straw, a

percentage which they regard as the normal nitrogen content both of faecal matter and of well-rotted farmyard manure. Of the total of 2 per cent., a maximum of 0.7 to 0.75 per cent. was shown to be held by the straw as ammonia. Any excess of ammonia present, or formed, which could not be utilized for the building up of the 1.3 per cent. of organically combined nitrogen, was readily lost by evaporation, especially on the drying of the manure heap.

The adjustment of the nitrogen content of the straw to 2 per cent. apparently takes place within the first four weeks of rotting. During the remainder of the ripening process, lasting from four to six months, the breakdown of the organic nitrogen to ammonia and the loss of ammonia by evaporation were found to keep pace with the loss of non-nitrogenous dry matter, thus maintaining the nitrogen percentage at 2 per cent. As the destruction of the dry matter ceased towards the completion of the rotting processes, the decomposition of the organic nitrogen and the evaporation of ammonia ceased, and the manure could consequently be kept for months without noticeable loss of ammonia. Any initial excess of nitrogen beyond 2 per cent. was found to be removed from the straw in the first four weeks of the rotting process by denitrification, either in the form of ammonia or as free nitrogen, without the straw becoming involved. To avoid such losses, Hutchinson and Richards advise the addition to a manure heap of sufficient quantities of straw to lower its percentage of nitrogen to 2 per cent.

From this outline of Hutchinson and Richards's interesting investigations it is clear that there are at least four important microbiological reactions involved in the preparation of farmyard manure, which in practice, and before fermentation, usually contains more than 2 per cent. of nitrogen. These comprise the elimination of excess nitrogen by denitrification, the oxidation of carbohydrate compounds resulting in the rise of the temperature of the manure heap, the stabilization of the nitrogen content to ensure an equilibrium between the organically held nitrogen and the nitrogen held as ammonia, and finally the conversion of the plant materials into *beurre*

*noir* or humic substances. To what extent do the cellulose and the hemicelluloses of the plant material, and to a lesser extent the pectin and the gum substances, take part in these decomposition processes?

From what has already been said it is clear that the denitrification processes resulting in the elimination of excess nitrogen as free nitrogen and ammonia do not involve the straw, but are performed by typical denitrifying and putrifying micro-organisms such as *Bact. fluorescens*, which converts organic nitrogen compounds into ammonia, even in the absence of carbohydrates (Emmerling and Reiser<sup>38</sup>).

The oxidation processes by which the temperature of the manure heap is raised have scarcely been studied since Dupont<sup>39</sup> undertook his investigations on the microflora of the aerobic fermentation of the manure heap in 1902. He came to the conclusion that *Bac. mesentericus ruber*, thriving well at a temperature of 50° C., and *Bac. thermophilus*, Grignoni, growing at temperatures up to 70° C., were the most important micro-organisms in this fermentation, and consequently responsible for the increased temperature. Of the two, *Bac. mesentericus ruber* fermented xylan readily, giving off carbon dioxide and forming small quantities of acetic and butyric acids, while *Bac. thermophilus*, Grignoni, showed little action on xylan, but fermented glucose, yielding carbon dioxide and appreciable quantities of acetic acid. That the microbiological decomposition of hemicelluloses, and particularly of xylan, gives rise to an increase in the temperature of accumulated vegetable tissues was also found by Mische<sup>34</sup> in the spontaneous combustion of hay. It is fairly safe to assume, therefore, that one of the functions of the hemicelluloses in the manure heap is to serve as a carbohydrate supply for those micro-organisms which bring about an increase in the temperature of the rotting vegetable material. That, however, is not their only function. Since many of the hemicellulose decomposers, and among them *Bac. mesentericus ruber*, convert organic nitrogen into ammonia, the hemicelluloses must take an active part in the second series of microbiological processes which is

responsible for the conversion of the manure nitrogen into a form which can be made readily available as a plant food. This has been regarded as the most important function of the hemicelluloses, but the losses which they suffer through this conversion are hardly large enough to justify that assumption. Thus Sjollem and Ruyter de Wild<sup>40</sup>, who investigated the metabolism of the pentosans during manure rotting, found that 2 kgs. of cow dung, subjected to aerobic fermentation at room temperature for two months, showed a loss of only 13 per cent. of its pentosans. Under these conditions the ammonia-producing and pentosan-decomposing micro-organisms must have developed well, though their growth, and consequently their enzymatic activity, might have been increased by a somewhat higher temperature. Nevertheless, the very large increase in the destruction of pentosans, amounting to a removal of 46.5 per cent. of the original total, which followed a change of the experimental conditions from aerobic to anaerobic, and an increase in the temperature of the reaction from ordinary room temperature to 35° C., points to the liability of the hemicelluloses to destruction by micro-organisms other than the aerobic ammonia producers. This view is confirmed by an examination of the decomposition products formed under anaerobic conditions in the manure heap. They include a considerable quantity of gas, mainly methane (Reiset<sup>41</sup> and Dehérain<sup>42</sup>), and thus indicate that the pentosans may take part in the decomposition processes which are often regarded as characteristic of cellulose. A good deal of further work is required to establish the extent to which the methane-producing cellulose-fermenting bacteria are capable of decomposing xylan. So far, Hoppe-Seyler's<sup>43</sup> preliminary experiments, in which he inoculated suspensions of xylan in water with river mud, are the only direct experiments available to support the view that xylan may be fermented by methane-producing cellulose-decomposing bacteria.

That hemicelluloses may be converted into organic acids, notably lactic acid, in the manure heap, still remains to be demonstrated. It appears likely, however, that they can be

decomposed in this way, since in the preparation of silage the pentosans are subjected in large measure to this type of decomposition. A conversion of hemicelluloses into organic acids would be of great value in the manure heap, since these acids would assist in retaining within the heap the ammonia formed from organic nitrogen.

The many reactions in which hemicelluloses, and particularly xylan, are involved during the ripening of the manure heap make the destruction of these substances a matter of primary importance, and there is a good deal of justification for Sjollem and Ruyter de Wild's statement that the value of a manure depends on the extent to which the pentosans have been decomposed. Nevertheless, their removal is in no case very complete, and fully 50 per cent. of the hemicelluloses originally present are left behind, even in well-rotted manure (König<sup>44</sup>).

Some of the micro-organisms which attack hemicelluloses in the manure heap are types which are capable also of decomposing pectin, notably the members of the *Bac. mesentericus* group. It is probable, therefore, that the pectin present will function on the lines of the hemicelluloses, that is, will assist in raising the temperature of the fermenting heap and in converting organic nitrogen into ammonia. The pectin may be decomposed also on the lines outlined when discussing the various retting processes, notably by members of the *Bac. amylobacter* type, which are found in large numbers in the soil and in the intestine of herbivorous animals (Henneberg<sup>10</sup>), whence they enter the manure heap. Knowing from Pringsheim's<sup>15</sup> investigations that these *Bac. amylobacter* forms are often capable of fixing atmospheric nitrogen when growing in symbiosis with cellulose-decomposing bacteria, it cannot *a priori* be excluded that processes may occur in the manure heap during its ripening, by which atmospheric nitrogen is fixed. This view is supported by Fulmer and Fred's<sup>45</sup> investigations on the nitrogen-fixing property of artificial mixtures of dung and wheat straw. It was found that a mannitol solution inoculated with such a mixture showed a distinct increase in total nitrogen content more marked at 28° C. than at 75° C. Pure cultures possessing the power of fixing nitrogen

under such conditions were isolated, and one of them, *Bact. azophila*, was described in detail by Fulmer<sup>46</sup>. Richards's<sup>47</sup> own investigations of the aerobic fermentation of the manure heap also support the view that nitrogen-fixing processes occur in the manure heap.

Hutchinson and Richards's observations that an artificial farmyard manure heap, prepared from straw and containing too little nitrogen before fermentation, increases its nitrogen content to the normal 2 per cent. by the absorption of ammonia from the surrounding atmosphere, does not, therefore, entirely exhaust the possibilities for the accumulation of nitrogen by the heap.

The decomposition of hemicelluloses and of pectin is probably to some extent also the work of actinomyces and of fungi, particularly in the compost heap. Both of these classes of micro-organisms have been isolated from the constituents of manure heaps. Actinomyces were obtained by Tsiklinsky<sup>48</sup> from faecal matter and by Miehle<sup>49</sup> from compost heaps. The presence of fungi in manure is discussed by Heneberg<sup>10</sup>. The extent of the activity of these organisms has not yet been measured, nor have the resulting decomposition products been established. From what is known of the physiology of the actinomyces it is probable that their destruction of hemicelluloses and of pectin is associated with a conversion of organic nitrogen into ammonia.

Even more extensive than the destruction of the hemicelluloses is the elimination of *cellulose* during the rotting of a manure heap. Though definite data are not available, an idea of the extent of this elimination can be obtained from Hébert's<sup>20</sup> investigations. This author found that 50 per cent. of the dry matter of a manure heap was lost after three months' fermentation. We may assume the original hemicellulose content of the straw composing the heap to be 20 per cent., that of cellulose to be 50 per cent., and that of lignin to be 20 per cent., and we know that 50 per cent. of the hemicelluloses of the straw would remain in the manure after rotting. It follows that some 50 per cent. of the cellulose, or two and a half times as much cellulose as hemicelluloses, would

have been decomposed during the three months' fermentation in Hébert's experiments, if the lignin and the remaining constituents of the straw had also been reduced by half, a reduction which is not likely to have been exceeded. In what manner, by what micro-organisms, and with what result is this large amount of cellulose decomposed?

From the earliest investigations dealing with the rotting of the manure heap this question has attracted wide interest, but its elucidation is still far from complete. This is not surprising, since the processes involved in the rotting of manure under agricultural conditions are complicated by various side reactions, notably by the metabolism of an excessive nitrogen content. Future investigations should be greatly simplified by Hutchinson and Richards's observations that a 2 per cent. nitrogen content reduces these complications to a minimum. The earliest investigations, notably those of Gayon<sup>50</sup> and Dehétrain<sup>51</sup> and his pupils, had shown that the rotting of the manure heap involves two separate fermentations, an aerobic heat-producing fermentation and an anaerobic methane- or sometimes hydrogen-producing decomposition. The anaerobic fermentation was found to be favoured by the high temperatures developed during the aerobic fermentation. As regards the nature of the responsible micro-organisms Gayon reported in 1884 that he had isolated in pure culture from a manure heap a 'small organism' which decomposed both cellulose and straw with evolution of methane. This observation was not confirmed by later investigators, however, and Löhnis and Kuntze<sup>52</sup>, who in 1908 endeavoured to isolate the cellulose-decomposing micro-organisms of the manure heap, failed completely in the task. If it is justifiable to judge by analogy, it is highly probable that the anaerobic cellulose decomposition processes of the manure heap are performed by such organisms as the thermophilic rod obtained by Fred, Peterson, and Viljoen<sup>17</sup> from horse dung, and the thermotolerant *Bac. cellulosaе dissolvens* of Khouvine<sup>18</sup> isolated from faecal matter of man. The latter organism may be particularly important in farmyard manure, since it is favoured in its growth by the presence of faecal matter. It is possible, of



course, that Omelianski's *Bac. methanigenes* and *Bac. fossilularum* may also play a part in the decomposition of the cellulose in the manure heap, but the high temperatures prevailing would probably militate against their development.

Since aerobic decomposition processes play an important part in the elimination of cellulose in the soil (van Iterson, jr.<sup>53</sup>), it is not unlikely that this reaction may also occur in the manure heap, especially in the compost heap, which is often loosely built up. Nor is it improbable that actinomycetes and fungi may assist in the disposal of the cellulose of the manure heap, just as they are probably taking part in the destruction of the hemicelluloses. The fungi are not likely, however, to exercise the dominating influence on the decomposition of the straw that Wehmer<sup>54</sup> is inclined to ascribe to them, at least not in the case of farmyard manure. For this to be the case their distribution in the heap would have greatly to exceed that indicated by the numbers actually found.

The microflora associated with the decomposition of cellulose in the manure heap is thus extremely varied. Its activity may result in the evolution of methane and hydrogen, the former, according to Dehérain<sup>42</sup>, when the reaction of the heap is neutral, and in the production of carbon dioxide and organic acids, compounds regarded as especially important by Dehérain and his pupils for the fixation of the ammonia formed from the decomposing nitrogen. Among other fermentation products may be mentioned the mucilaginous substances found in pure culture of many cellulose fermenters. This mucilage is perhaps present in the plastic substances to which plant tissues are reduced in well-rotted farmyard manures.

In 1922 Henneberg<sup>10</sup> attempted to study the microflora of a rotting compost heap by direct examination. The results contain little of interest. They confirm the view, however, that the preponderating microflora belongs to the bacteria and not to the fungi.

In this outline of the breakdown of cellulose in the manure heap sufficient stress has not, perhaps, been laid on the fact that the bulk of the cellulose is present as ligno-cellulose, a substance which is regarded as being less readily decom-

possible by bacteria than is pure cellulose. The complication introduced by this fact is still too little understood for its effect on the composition of the active microflora to be estimated. It raises, however, the important question of the ultimate fate of the lignin content of the manure heap. Here observations are, practically speaking, limited to the vague statements made by the early investigators, who reported (Hébert<sup>55</sup>) that part of the lignocellulose, or vasculose, became dissolved in the alkaline liquid of the heap and thus formed the *matière noire*, the chief constituent of manure—its content of humic substances.

This introduces the highly debatable subject of the origin and the nature of the humic substances—sometimes termed ulmins (see Stopes and Wheeler<sup>56</sup>).

By the expression humic substances, or ulmins, is understood the brownish or almost black degradation product of plant tissues, soluble in ammonia and other alkalies, and precipitated as a gel-like deposit from these solutions by the addition of an excess of acid. This property of the humic substances of dissolving in alkalies and of being precipitated on subsequent neutralization of their solutions with acids has given rise to the belief that they have an acidic nature. They are therefore spoken of as humic acid and humic salts respectively. In spite of the fact that blue litmus is turned red by humic acid, Baumann and Gully<sup>57</sup> deny that it has a true acidic nature. These authorities regard it as a colloid, in which organic acids and salts are held by absorption.

Humic substances may be formed naturally by microbiological decay, for instance in the manure heap and by the decay of vegetable matter in the soil, or they may be artificially prepared by the action of mineral acids on carbohydrates or by the oxidation of phenols. Their chemical composition varies considerably. Speaking broadly the natural humic substances contain carbon, hydrogen, oxygen, and nitrogen, while those formed artificially from carbohydrates contain only carbon, hydrogen, and oxygen. The mother substance may be aliphatic compounds such as carbohydrates, aromatic compounds such as phenols, or pectin, or protein decomposition products, either alone (Schmiedeberg<sup>58</sup>) or in combination with

carbohydrates (Jodidi<sup>59</sup>). The humic compounds derived from proteins and protein decomposition products are termed melanoids by Schmiedeberg. They will not be dealt with here.

As regards the humic substances derived from aliphatic and aromatic compounds it has now been established, thanks to the researches of Eller and his collaborators (<sup>60, 61, 62</sup>), that they fall within two clearly defined groups, the first of which comprises the humic substances formed naturally by the decay of vegetable tissues and those prepared artificially by the oxidation of aromatic compounds such as phenols. The second group comprises the humic substances prepared artificially by the action of mineral acids on carbohydrates. It may be added here that humic substances may also be formed from carbohydrates (pure cellulose) which have been kept for many centuries, without exposure to microbiological decomposition, for instance in mummy cloth. Further reference to work on this humus is made in Chapter X in discussing the question of the formation of peat and coal.

From his researches Eller comes to the conclusion that the humic substances prepared from a sample of lignite investigated by him, and from phenols, are identical in their essential features. The humic compounds prepared from pyrogallie acid in the presence of an alkali were found to have the formula  $(C_{48}H_{30}O_{25})$ ; 95 per cent. of the lignite used consisted of exactly the same substance, and showed signs of having been formed, in part at least, from decayed tree trunks, that is, from material which may have been rich in lignin. This statement by Eller defines in so many words the problem, at present so widely discussed, of the nature of the mother substance of naturally formed humic substances, and of whether peat and coal have been formed chiefly from lignin, or from cellulose, or from a mixture of both. In any case, Eller's investigations, as well as those of Fischer and Schrader<sup>63</sup>, have made it clear beyond doubt that lignin takes an important part in the formation of natural humic substances. This view had been already expressed by Hébert<sup>29</sup> and before him by Fremy<sup>64</sup>, and supported by many other investigators, including

Hoppe-Seyler<sup>43</sup>, who was unable to trace humic substances in the products of the decomposition of pure cellulose by micro-organisms. Hoppe-Seyler's observations have frequently been confirmed, most recently by Wehmer<sup>54</sup>, and are undoubtedly correct. On being decomposed by pure cultures of micro-organisms pure cellulose does not yield humic substances.

(2) **The microbiological decomposition of cellulose and hemicelluloses on the surface of and in the soil.** Wherever vegetable debris accumulates in shallow layers, as for instance on the floor of forests, the microbiological decomposition of its cellulose and hemicelluloses and the consequent production of humus must be considerably influenced by the fact that free access of oxygen is possible throughout the layer, and that the possibility of any appreciable increase in the temperature of the debris is excluded. Neither anaerobic nor thermophilic micro-organisms are likely, therefore, to play the important role which they undoubtedly perform in the fermentation process of the manure heap. On the other hand, the rotting of vegetable matter on the forest floor, and in other places where a shallow accumulation occurs, offers special facilities for the development of fungi, and in the past these micro-organisms, as well as actinomycetes, have been regarded as the chief rotting agents under such conditions. Data referring to the relative importance of fungi and bacteria in the decomposition of shallow layers of vegetable matter were collected by Ramann, Remelé, Shellhorn, and Krause<sup>66</sup>, who found that the decaying leaf-covering of a pine wood which had an undergrowth of beech showed 1,000 bacteria to 1 fungus. In the decayed layer, i. e. in the mould, the ratio was 100 bacteria to 20 fungi. In the soil immediately under the mould there were 100 bacteria to 257 fungi, and in the actual surface soil 100 bacteria to 171 fungi. Taking the acidity of the decaying matter as 0, that of the mould was found to be 0.65 to 0.98, the acidity of the top soil and mould mixture 0.251, and that of the surface soil 0.007. Where the decaying debris of a pine wood was covered by moss and lichen the following ratio of bacteria to fungi was found: in the mould 100 bacteria to 373 fungi, and in the soil immediately underneath 100 bacteria

to 339 fungi. Here the acidity of the mould varied, and was in one case 0.579 and in another 0.160. In the samples taken from the surface soil the acidity in the two cases was 0.005 and 0.016 respectively. Where the debris of a forest soil was rich in chalk the ratio was found to be, in the mould 1,000 bacteria to 14 fungi, and in the soil immediately underneath 10 bacteria to 33 fungi. Though the soil in this case was neutral, it nevertheless contained three times as many fungi as bacteria. That fungi are more active in the disposal of cellulose in the soil than bacteria and actinomycetes is also asserted by Henkelekian<sup>66</sup>. It should not be overlooked, however, that the bacteria considered in these experiments were representatives of a secondary microflora, that is of such forms as develop on ordinary laboratory media. No account was taken of any of the numerous types which do not thrive under such conditions, notably of the aerobic cellulose-decomposing bacteria.

The rate of decay of vegetable debris has been studied by Kostycheff<sup>67</sup>, who observed a loss of 55 per cent. of dry matter in one year and 73 per cent. in two years, figures which were confirmed by Henry<sup>68</sup>.

As to the resulting decomposition products, the literature contains statements by Pierre<sup>69</sup>, Müntz<sup>70</sup>, and by Stoklasa and Ernest<sup>71</sup> indicating that ethyl alcohol, acetic acid, butyric acid, and carbon dioxide may be formed under such conditions, or at least during the decomposition processes occurring in soil rich in organic matter. The presence of these decomposition products speaks for a considerable measure of activity on the part of bacteria.

A study of the decay of vegetable debris on the forest floor from the point of view of the formation of humus was undertaken by Koning<sup>72</sup>, partly in collaboration with Oudemans. Koning expresses the view that hyphomycetes are far more important in this respect than bacteria. The conversion of leaves into mould is ascribed by him in part to the activity of lower animals, a view previously expressed by Darwin<sup>73</sup>, for instance nematodes, lumbricides, arachnides, myriapodes, &c., which disintegrate the debris and distribute it evenly

throughout the surface layer of the soil. The chief decomposition, however, according to Koning, is performed by fungi. Even while living, Koning states, the leaf possesses a specific flora of fungi, which is sufficiently characteristic to indicate the age of the leaf, since it changes as the leaf grows older. Of the individual types of this flora Koning emphasizes the importance of *Trichoderma Koningii*, Oudemans, which he finds active during the whole process of humification. It is said to attack both pectin and cellulose.

Other cellulose-decomposing fungi have been isolated from soil by van Iterson, jr.<sup>53</sup>, who mentions among others *Sordaria humicola*, Oudemans, *Pyronema confluens*, Tulasne, *Chaetomium Kunzeanum*, Zopf, *Botrytis vulgaris*, Fries, *Stemphylium macrosporoides*, Saccardo, and *Cladosporium herbarum*, (Persoon) Link, as special cellulose-decomposing soil inhabitants. Speaking broadly, it is probable that most wood-destroying fungi are able to take part in the decay of vegetable debris accumulated on the surface of the soil, and can thereby assist in the formation of humus. It is disappointing, however, that neither the statements of Koning, nor those of any other investigators on the importance of fungi in humification processes, are substantiated by convincing experimental evidence. The nearest approach to such evidence was brought forward by Wehmer<sup>54</sup> in a recent paper on the conversion of wood and cellulose to humic substances by fungi.

To some extent this lack of definite experimental evidence is due, no doubt, to the overrating of the importance for the humification processes of some micro-organisms, and probably also to the underrating of the significance of others, notably of the aerobic cellulose-decomposing bacteria. It is hardly justifiable, for instance, to measure, as Rullmann<sup>74</sup> did, the importance of an actinomycete in the formation of humus by the similarity in the odour emanating from its cultures to that issuing from mould and soil on damp warm days in spring. Nor was Beijerinck<sup>75</sup> justified in gauging the importance of another actinomycete by its property of forming quinone. Wehmer, in his investigation referred to above on the importance of fungi for the humification processes, appears

to have overlooked the existence of aerobic cellulose-decomposing bacteria.

The chief reason for the lack of definite information on the origin of humus must no doubt be ascribed to the obscurity of the chemistry of lignin and to the ignorance of the action of micro-organisms on this substance. Wehmer obviously realized this, since he endeavoured to determine the action of fungi on lignin freed from cellulose. Judging by his results, his work was probably carried out with a substance which had had its chemical structure altered during preparation. Once the chemistry of lignin is understood, or at least when it is possible to prepare a pure lignin without any alteration of its structure, and once the extent is realized to which the various classes of micro-organisms are able to decompose lignin and the degree to which they are inhibited or accelerated in their growth by its presence has been established, then will it be possible to throw light on the question of the participation of micro-organisms in the formation of humus and on the relative importance of cellulose and lignin for this process.

The earliest workers on the decomposition of cellulose *in* the soil were well aware of the immense importance of the subject. They were debarred from subjecting the processes involved to a serious study, however, by the difficulties involved in the isolation of the responsible micro-organisms. Surveying the subject of the decomposition of cellulose in the soil on the basis of present knowledge, it must be confessed that these difficulties are still a serious barrier against progress. It is true that Omelianski succeeded in isolating two anaerobic cellulose-decomposing bacteria and that van Iterson opened the eyes of the microbiologist to the importance of the aerobic cellulose-decomposing bacteria, of which a type has occasionally been isolated in pure culture. But the fact remains that even to-day investigators of cellulose destruction in the soil are compelled to study this process under conditions complicated by all sorts of side reactions which take place in the soil as a direct or indirect result of the breakdown of the cellulose. In the face of these difficulties what, then, has hitherto been achieved?

It has been established (van Iterson, jr.<sup>53</sup>) that both fungi and bacteria take part in the destruction of pure cellulose in the soil, the former particularly when the reaction of the soil is slightly acid. Bradley's<sup>76</sup> investigations have illustrated the wide distribution of cellulose-decomposing bacteria. The work of Kellermann and his collaborators<sup>77, 78</sup> has made it highly probable that aerobic bacteria are more important in the disposal of cellulose in the soil than anaerobic forms. Groenewege<sup>79</sup> has shown that the elimination of the cellulose may be responsible for the destruction of nitrates, owing probably (Rippel<sup>80</sup>) to secondary reactions set up by denitrifying micro-organisms, which utilize the hexoses liberated during the destruction of the cellulose. Fringsheim<sup>15</sup> has found that atmospheric nitrogen may be fixed by similar symbiotic reactions. Finally Charpentier<sup>81</sup> and Bartel and Bengtsson<sup>82</sup> have brought evidence to show that the presence of nitrogen, notably in the form of ammonia, facilitates the disposal of cellulose in neutral and alkaline soils. The beneficial action of farmyard manure on the decomposition of cellulose in the soil would appear to be explained by the work of the two last-named authors. That lignocellulose, in the form of straw for example, may replace pure cellulose in all the above reactions is made clear from Starkey's<sup>83</sup> investigations, which showed that 20 per cent. of the rye straw introduced into a fertile soil was decomposed in ten days: in acid soils the decomposition was less rapid. This observation that the destruction of lignocellulose, and presumably of cellulose, proceeds more slowly in acid soils than in soils of neutral reaction is of great importance in the interpretation of the origin of peat.

In well aerated soils the microbiological decomposition of vegetable debris is sufficiently rapid to prevent an accumulation of organic matter, and it has frequently been established that the percentage of organic matter in arable soils remains low in spite of the annual introduction of large quantities of dead vegetable matter. Thus Cameron<sup>84</sup> found an average of only 2.06 per cent. of organic matter in 1,340 samples of American soil. From this it is clear that the various micro-



biological processes in which cellulose and lignocellulose become involved when introduced into the soil must eliminate immense quantities of these substances. Not even the humic compounds resulting from the decay of vegetable debris are exempt from destruction in the soil. Thus Agafonoff<sup>85</sup> found only 2.92 per cent. of humus in the soil formed on an ancient Gallo-Roman wall which must have been about 2,000 years old. But in spite of the fact that humus is thus naturally decomposed, it has nevertheless not been found capable of supporting microbiological growth when used as a food substance in purified form (Koning<sup>72</sup>), or at least has not been found capable of serving as a carbohydrate supply for actinomycetes and fungi, which are the micro-organisms regarded by Nikitinski<sup>86</sup> as being especially adapted for the decomposition of humus. A renewed study of the reason for the unsuitability of purified humus as a food substance, as well as of the whole question of the microbiological decomposition of natural humus would amply reward the investigator for his efforts.

The fate of hemicelluloses in the soil is probably intimately bound up with that of the decomposition of cellulose. Like cellulose, they undoubtedly assist both in the liberation and in the fixation of nitrogen. That they are involved also in the conversion of organic nitrogen into ammonia is clear from the presence in most soils of members of the ammonia-forming and hemicellulose-decomposing group of *Bac. mesentericus*. It is also noteworthy that the acid-producing hemicellulose-decomposing bacteria studied by Pringsheim<sup>3</sup> were obtained from soil and that *Bac. acetolactylicus* used by Northrop and his collaborators<sup>87</sup> for the conversion of xylose into acetone and ethyl alcohol was probably a soil inhabitant, since it was first obtained from decaying potatoes. From these facts it is reasonable to assume that the decomposition of hemicelluloses in the soil may lead, directly or indirectly, to the formation of both organic acids and alcohols.

Whether hemicelluloses take part in the formation of humus is still a debatable question. Trusov<sup>88</sup> in a recent paper denies this. Suzuki<sup>89</sup> on the other hand finds that they do, a view

which is supported to some extent by the analytical data of Michelet and Sebelien<sup>90</sup>, who found 3.69 per cent. of pentosans and 1.74 per cent. of methyl pentosans in a humus preparation obtained from a Norwegian agricultural soil.

(3) **The microbiological decomposition of cellulose and hemicelluloses under water.** The problem of the decomposition of cellulose and hemicelluloses under water is at least equal in importance to that of their destruction in the soil. In lakes, marshland, and canals, in tropical climates, in mangrove swamps, and in the sea of all climates, tremendous quantities of vegetable debris composed largely of cellulose or hemicelluloses accumulate and decay, chiefly through microbiological activity. Very little is known of the reactions involved, except that marsh gas is frequently formed as a result of the decay. This gas at first remains entangled in the mud of the water bed, but eventually rises to the surface of the water, either as bubbles or sometimes in a continuous stream. It is this gas which, when becoming spontaneously ignited, was known of old as the will-o'-the-wisp.

The decay of vegetable matter under water is in large measure the result of the activity of anaerobic micro-organisms and may be reproduced in the laboratory under anaerobic conditions, as was shown by Popoff<sup>91</sup> and by Hoppe-Seyler<sup>92</sup>. It was from mud of the river Neva that Omelianski<sup>93</sup> obtained the first two anaerobic cellulose-fermenting bacteria to be isolated, *Bac. methanigenes* and *Bac. fossicularum*. Their action on vegetable tissues decaying under water is evidence that organic acids, notably acetic and butyric, are formed under such conditions. The influence of these acids on the progress of the decomposition can at present be visualized only in its broadest outlines. They become neutralized and are consequently unable to impede the decomposition where alkaline reacting substances are present in the mud or in the surrounding water. When neutralized they may possibly be decomposed to methane and carbon dioxide by micro-organisms of such a type as that described by Mazé<sup>94</sup>, and isolated by him from a crude culture of cellulose-decomposing bacteria. In running water they will be removed

partly by neutralization and partly by dilution. Under conditions where a neutralization or an elimination by dilution is excluded, for instance in stagnant water, their action on the progress of the decomposition must be considerable. That this is so can be gauged from laboratory experiments, where the decomposition of cellulose by *Bac. methanigenes* or *Bac. fossicularum* comes to a premature standstill if the acidic decomposition products are not rendered innocuous by neutralization. Where vegetable debris becomes deposited in stagnant water which for some reason or other has an acid reaction, for instance in many peat bogs, its destruction by micro-organisms is therefore likely to remain incomplete unless other factors take part in the disintegration.

That the decay of cellulose and hemicelluloses under water is not entirely an anaerobic process is clear from Issatchenko's<sup>95</sup> investigations. This author recently isolated two aerobic cellulose-decomposing bacteria from the medicinal mud of Lake Saki (Crimca). The organisms are stated to resemble those obtained by van Iterson, jr., and are thus, probably, similar to *Spirochaeta cytophaga*. Both are claimed to take an active part in the formation of the medicinal slime or mud for which this lake is renowned. When grown in pure culture one of the organisms shows a whitish, and the other a yellowish growth, and both give rise to the formation of copper-reducing substances (cellobiose?).

Technically the decomposition of cellulose under water is utilized in sewage works, for the disposal of paper and similar cellulose-containing solid matter. In the septic tank this material is allowed to settle and subsequently undergoes a fermentation process resulting in its conversion into soluble and gaseous products. A detailed study of the microbiological reactions thereby involved has not yet been carried out.

(4) **The intestinal decomposition of cellulose and hemicelluloses.** Pasteur<sup>96</sup> expressed the view in 1885 that micro-organisms would one day be found as important in the normal digestive processes of the animal intestine as Duclaux<sup>97</sup> had shown them to be in the preparation of suitable plant foods in the soil. Ten years later Nuttall and Thierfelder<sup>98, 99, 100</sup> under-

took to test Pasteur's suggestion experimentally. For this purpose and following Pasteur's advice they reared aseptically born guinea-pigs for eight days under sterile conditions, feeding the animals on sterile cow's milk, either alone or in a mixture with sterile biscuits. The animal fed exclusively on cow's milk developed well, while the other showed a markedly smaller gain in weight than normal guinea-pigs of the same age. The slower growth of the animal fed on the mixed diet was ascribed by Nuttall and Thierfelder to a constitutional damage caused by the artificial method of birth and not to the absence of micro-organisms in the intestine. In fact Nuttall and Thierfelder concluded from their experiments that micro-organisms were unnecessary for the normal development of the guinea-pig. That the guinea-pigs when weaned, and indeed all herbivorous and omnivorous animals, require a diet consisting chiefly or entirely of plant tissues, appears to have been completely overlooked by them.

Nuttall and Thierfelder's conclusions did not long remain uncontradicted. In 1899 and subsequent years Schottelius<sup>101, 102, 103</sup> gave an account of his classic experiments on the rearing of sterile chickens, a type of animal far better suited than the guinea-pig for the study of the importance of micro-organisms for the digestion of vegetable tissues. His experiments led him to the conclusion that the presence of micro-organisms in the intestine is absolutely necessary for the normal development of the chicken, and that the microflora of the digestive tract assists in the resolution of the food consumed. Schottelius's view is now generally accepted as true, at least for most herbivorous and omnivorous higher animals, and in so far as this flora supplies enzymes which are essential for the digestion of the food and are not produced by the animal itself. It should be pointed out, however, that Metchnikoff and his collaborators<sup>104</sup> have demonstrated that species of mammals exist, such as the large fruit-eating bat (*Pteropus medius*), in which the microflora of the intestine is numerically insufficient to take part in the digestion of the food. But in such cases the amount of faecal matter produced is very high in proportion to the amount of food consumed.

In 1882 — long before Schottelius's investigations — Tappeiner<sup>105</sup> had associated micro-organisms with the intestinal decomposition of cellulose, a name which at that time comprised cellulose, hemicelluloses, and pectin. From his investigations of the gases produced in the alimentary canal Tappeiner came to the conclusion that cellulose may be decomposed during digestion by two different types of organized ferments (micro-organisms), one active at alkaline reactions and yielding hydrogen and carbon dioxide, and the other producing methane and carbon dioxide at acid reactions. In both cases fatty acids, notably acetic and butyric acids, were found by Tappeiner as further decomposition products, and from this he concluded that the nutritive value of cellulose was limited to the value of these fatty acids. Of the two fermentations, Tappeiner regarded the methane fermentation as the dominating cellulose decomposition process, occurring chiefly in the paunch and to some extent also in the large intestine and in the lower part of the small intestine, of those ruminants which he investigated.

The efforts of workers in this field during the three decades following Tappeiner's publications were centred on deciding the question of whether micro-organisms alone are responsible for the digestion of cellulose in the intestine, or whether they do so in collaboration with enzymes secreted by the alimentary canal, on determining the seat of this decomposition, and on measuring the nutritive value of cellulose. That the decomposition of cellulose is due solely to the activity of micro-organisms can now be regarded as definitely proved, thanks chiefly to the investigations of Ellenberger and his collaborators<sup>106</sup>, of Scheunert<sup>107</sup>, and von Hoesslin and Lesser<sup>108</sup>. Though enzymes are not, therefore, to be regarded as mainly or solely responsible for the digestion of cellulose, as had been suggested by Holdefleiss<sup>109</sup>, it must be admitted that they may have an action on cellulose. Thus Knecht<sup>110</sup> observed that bleached cotton hairs soaked for some time in human saliva retained aniline dyes better than untreated hairs.

The seat of cellulose fermentation in herbivorous and omnivorous animals has been allocated by Ellenberger and his

collaborators to the paunch (where present), to the caecum, and, to some extent, to the ilium and the colon.

The work carried out to determine the nutritive value of cellulose has established that it is almost equal in this respect to starch, provided it is not present in heavily lignified form, such as winter straw or wood (Kellner and others<sup>111</sup>). In the case of crude fibres such as winter straw and wood, the material requires a preliminary digestion with alkali, a treatment which was of great practical importance in Germany during the world war (Pringsheim<sup>13</sup>). From the fact that cellulose under favourable conditions possesses a high nutritive value, it is clear that its utilization by the animal is independent of the fatty acids produced during its decomposition, and is probably due, as has been suggested by Pringsheim and by Khouvine<sup>16</sup>, to the formation of soluble carbohydrates such as cellobiose and glucose as decomposition products.

While the problem of the digestion of cellulose has thus been made considerably clearer in several directions, the question of the micro-organisms engaged in its decomposition is almost as unexplored to-day as it was at the time of Tappeiner's publications. This is due to the many difficulties which are met with in attempting to isolate these micro-organisms. It must be regarded as an important step forward that Henneberg<sup>10</sup>, in order to avoid these difficulties, recently undertook to study the intestinal flora microscopically, especially from the point of view of the destruction of cellulose, hemicelluloses, and pectin.

On closer consideration such investigations as Henneberg's may be found to give a somewhat distorted picture of the microflora engaged in the decomposition of vegetable tissues in the intestine. But their value is not to be sought in a correct enumeration of the various intestinal cellulose-decomposing micro-organisms, but rather in the stress they lay on the benefit which the investigators of this problem may derive from a microscopic examination of the intestinal content, particularly of the vegetable tissues in process of resolution. In such tissues certain types of micro-organisms are often found in almost pure culture and under conditions

which make it possible to draw conclusions as to the part they play in the resolution of the vegetable tissue. More care than is shown by Henneberg is necessary, however, in framing conclusions. It is thus hardly justifiable to conclude that one of the organisms described as a cellulose decomposer is so because it is found in considerable numbers in cavities produced by the activity of its enzymes in vegetable tissues such as bean pods, which are known to be composed largely of hemicelluloses (Schulze and Pfenninger<sup>112</sup>). The fact that in its morphology, its staining reactions, and its property of fermenting starch it resembles the pectin-decomposing *Bac. amylobacter* group should have been sufficient warning against describing it as a cellulose decomposer without further evidence. It should be mentioned also that Henneberg's statement that most of the cellulose-decomposing organisms found in the intestine are iodophil, that is, can be stained blue or purple with iodine, disagrees with all previous observations of the staining properties of cellulose-decomposing micro-organisms. Thus, Omelianski<sup>20</sup> asserts in his account of *Bac. methanigenes* and *Bac. fossicularum* that these types are not stained blue or purple by iodine, a point which he emphasizes as particularly important.

Nevertheless, Henneberg's method of analysis is undoubtedly a very valuable one when used with discretion, and the microscopical study of the intestinal content of a cellulose-digesting animal fed on fibres of pure cellulose would do much to enrich existing knowledge of the microflora responsible for cellulose decomposition in the intestine. The observations so far made on the subject may be summarized by stating that tissues entering the alimentary canal are attacked by a large variety of micro-organisms, including actinomycetes, some of which can be recognized as pectin decomposers, and others, for instance the *Bac. mesentericus* forms, as hemicellulose fermenters. The presence of types resembling *Bac. methanigenes* has also been established.

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## CHAPTER IX

# ENSILAGE AND SPONTANEOUS HEATING OF PLANT MATERIALS

**Preparation of silage.**—In many localities where green fodder cannot be converted into hay and where root crops are difficult to procure, a succulent cattle food may be prepared by converting the green fodder into silage. In the process of ensilage the green fodder is stacked or placed in a pit, either whole or after preliminary cutting, and is then allowed to undergo a spontaneous fermentation in the absence of oxygen. To prevent the access of oxygen the plant material is compressed after stacking, either by mechanical means or by loading with a layer of soil. This pressure is sufficient to cause the water contained in the plant material to penetrate the whole stack or pit uniformly and to render the green fodder water-logged. This, in addition to the absence of oxygen, ensures that the fermentation proceeds on the desired lines and is completed before the cellulose and most of the hemicelluloses of the plant tissues have been destroyed. This is essential, since it is largely on the content of these polysaccharides that the nutritive value of the finished silage depends. Judging from Hansson's<sup>1</sup> investigations, both the cellulose and hemicelluloses appear to be acted upon during the fermentation, since badly or insufficiently fermented silage was found by him to have a much lower nutritive value than properly fermented silage.

During fermentation the temperature of the plant material rises somewhat, especially when oxygen gains access. In such cases temperatures of 45 to 50° C., or even higher, may

be reached (Griffiths<sup>2</sup>), but these are undesirably high. Normally the fermentation is conducted between 25 and 45° C.

Depending on the condition of the plant material used and also on the method of its packing, Amos and Williams<sup>3</sup> distinguish five types of silage.

*Sweet silage* is of a dark brown colour, and is obtained by loosely stacking wet or fairly fresh material in comparatively shallow layers. In such material oxygen is not entirely excluded, and the temperature, therefore, rises during the ensuing fermentation to 45 or 50° C. This type of silage, though palatable, is stated to have a comparatively low feeding value owing to the loss of nutrients incurred through the excessive heating.

*Acid, light brown, or yellow-brown silage* is formed when the temperature is maintained between 30 and 37° C. by packing the material more closely and in thicker layers. The crops used for this type of silage should be moderately mature, and so far wilted that their moisture content amounts to about 70 per cent. This type of silage is eaten greedily by stock and has a high nutritive value.

*Green fruity silage* is produced in tower silos from crops cut in the earlier stages of maturity, that is, at the time of flowering or of seed formation. The plant material is ensilaged before withering and is packed closely to prevent the temperature rising higher than 34° C. during fermentation. This silage is relished by stock and has a high digestibility. On account of its freshness, however, a considerable amount of its feeding value is lost in the liquids draining off during and after fermentation.

*Sour silage* is prepared from immature and succulent plant material, or from crops which have been heavily soaked by rain after cutting. It is not a particularly good cattle food.

*Musty silage*, a type of silage in which fungi would appear to have largely replaced bacteria during fermentation, is of little or no value as a cattle food.

The time taken to convert green plant material into these various types of silage is not given by Amos and Williams, but judging by the work of other investigators ensilaging takes from 30 to 50 days to complete.

As may be gathered from the above account of Amos and Williams's work, the finished silage may have either a neutral or an acid reaction, depending in large measure on the amount of oxygen which gained access to the plant material

during fermentation. The neutral or slightly acid types consist of wet, brown, or almost black, compounds, still retaining the complete structure of the now brittle plant tissues of which they are composed. In appearance they strikingly resemble samples of young peat. Both the sweet and the acid types of silage may be preserved unimpaired for years without undergoing further decomposition, always provided that oxygen is excluded.

In addition to the cellulose and the hemicelluloses, of the latter of which about 15 to 20 per cent. is destroyed during fermentation (Peterson, Fred, and Verhulst<sup>4</sup>), silage retains the lignin content of the original material, though perhaps partly converted into humus, the presence of which was reported by Russell<sup>5</sup> in the samples of silage studied by him.

The original nitrogen content, though probably not diminished quantitatively, undergoes a decomposition during ensilage by which part of it becomes converted into peptones and amino-acids. Swanson and Tague<sup>6</sup> found about one-third of the original protein converted into amino-acids in silage prepared from lucerne, and Edin and Sandberg<sup>7</sup> found that 10 to 20 per cent. of the total nitrogen of good silage was present as ammonia, and 22.8 to 45 per cent. as amino-acids and peptones.

The soluble carbohydrates originally present in the plant material are lost during ensilaging according to Russell<sup>5</sup> and to Lamb<sup>8</sup>, and any starch present probably disappears also. In their stead pentoses are formed from the pentosans and other hemicelluloses, and these may be found in the finished silage (Peterson, Fred, and Verhulst<sup>4</sup>). Most silage samples contain small amounts of alcohols, the nature of which has not been definitely established. They are regarded generally as consisting principally of ethyl alcohol, though it is probable, as Burrill<sup>9</sup> suggests, that higher alcohols are present also. Even acetone may perhaps be present, since some pentose-fermenting bacteria, e. g. *Bac. acetoethylicus*, Northrop<sup>10</sup>, produce this substance.

The alkali solubility of the finished silage is very high. The dark brown colour can be largely extracted by boiling

silage with dilute solutions of an alkali. On neutralization of this solution a precipitate is formed showing the properties and characteristics of natural humic compounds (Russell<sup>5</sup>).

The fermentation process to which plant material is subjected during ensilage has been variously ascribed to the action of enzymes produced by the living cells of the plant tissues and to the activity of micro-organisms, and a good many investigations have been carried out to settle the relative importance of the two agencies since the date when the preparation of silage became an important agricultural process. The earliest workers, among them Burrill<sup>9</sup>, Griffiths<sup>2</sup>, and Emmerling<sup>11</sup>, were on the whole inclined to accept the view that micro-organisms were entirely or at least chiefly responsible for the changes in the plant material undergoing ensilage. But subsequent work, for instance that of Russell<sup>5</sup> and of Samarani<sup>12</sup>, ascribed all the essential changes to the action of the plant enzymes present in the accumulated tissues and allotted to micro-organisms only a purely secondary role. During the last fifteen years or so this view has again been abandoned in favour of an explanation which concedes to micro-organisms an important part of the reactions, while still allowing that under normal conditions plant enzymes may be active during the early stages of fermentation. This view is adhered to by Jones and Gibbard<sup>13</sup> and by Lamb<sup>8</sup>, among others. Jones and Gibbard, who undertook a study of the changes occurring in the numbers of micro-organisms present in sweet clover undergoing ensilage, found that the bacteria developing on ordinary agar increased in number from 30 millions per gramme of material before fermentation to a maximum of 260 millions on the fourteenth day of ensilaging. Subsequently their numbers again decreased. The increase was found to be chiefly due to an increase in the acid-producing and acid-tolerant types, such as *Bact. bulgaricum* and related organisms, the number of which rose from 9 millions per gramme to 200 millions per gramme on the fourteenth day. The gelatine-liquefying forms, regarded by Jones and Gibbard as identical with putrefying types, decreased steadily in numbers during ensilaging from

8 millions to half a million on the twenty-first day of the fermentation.

In most of the silage samples examined Jones and Gibbard found no yeasts. In some samples yeasts were present in small numbers, the largest being 110,000 per gramme of silage on the third day of fermentation. After the tenth day no yeasts could be found in any of the samples. This observation is interesting in view of Esten and Mason's<sup>14</sup> statements that large numbers of yeasts are found in silage and that they are responsible for the production of at least part of the alcohol present, a view which has been accepted by Lamb as late as in 1925. Hunter<sup>15</sup>, on the other hand, had emphasized a few years before Jones and Gibbard's investigations were published that the yeast found in silage took little part in the fermentation, except perhaps during the first two days. And Burrill<sup>9</sup>, in one of the earliest studies on ensilaging, remarks that the yeast found in silage does not produce alcohol, but belongs to the *Mycoderma* group which oxidizes organic acids.

Fungi were very sparsely represented in the silage samples investigated by Jones and Gibbard. While no less than seventeen species of such micro-organisms were isolated from the clover material used, only one, *Oidium lactis*, was found in the fermenting samples, except in the case of the top layer of the heaps, which could not be regarded as typical. The highest numbers of *Oidium* were registered between the fifth and the tenth day of fermentation, after which they gradually decreased until by the twentieth day the fungus was only found in two samples.

Large numbers of micro-organisms have also been reported present in silage by Sherman<sup>16</sup> and by Lamb<sup>8</sup>, the latter recording an increase of twelve times the original number during the first nine days of fermentation. Lamb's investigations, which were undertaken to determine the relative importance of micro-organisms and plant enzymes for the conversion of green fodder into silage, are particularly interesting because of the unorthodox manner in which the solution of this problem is sought. Instead of studying the

fermentation on material in which the plant enzymes had been previously destroyed, or on material rendered sterile by the addition of antiseptics, the two standard methods hitherto used for the study of the silage fermentation, Lamb determines the ratio of production of several of the decomposition products formed during the fermentation, and compares their curves with the similarly constructed reaction curves of typical plant enzymes and of those of typical microbiological reactions, on the basis of Rahn's<sup>17</sup> observations on such curves. This comparison leads Lamb to the conclusion that micro-organisms are chiefly responsible for the production of acids and alcohols, and the consequent disappearance of soluble carbohydrates, though he concedes that some alcohol may be formed by plant enzymes during the early stages of the ensilage process. The protein of the plant material, according to Lamb, is first attacked and hydrolysed by plant enzymes, a reaction which is continued later by micro-organisms. The evolution of carbon dioxide is a joint action of plant enzymes and of micro-organisms, while the latter are chiefly responsible for the rise in temperature of the fermenting plant material. The views of Jones and Gibbard, and of Lamb, that micro-organisms are largely responsible for the changes, appear to be entirely justified, when their number and variety, and their increasing development during fermentation are taken into account.

Though the question of the relative importance of plant enzymes and micro-organisms may now be regarded as settled, there are still many microbiological problems to be solved in connexion with the preparation of silage. It is still necessary to establish, for instance, what changes are suffered by the cellulose, the pectin, and the gums present, a subject on which nothing is known. The function of the hemicelluloses must also be more thoroughly investigated and a detailed analysis undertaken of that part of the microflora which does not develop on ordinary laboratory media. Even with these questions answered there still remains unexplained the increased feeding value of the cellulose of the plant tissues after fermentation, a problem which may be connected with



changes in the lignin present. Further work is also required to determine whether nitrogen is lost during the fermentation, a question raised by Emmerling<sup>11</sup>, and, if so, by what means such loss can be prevented.

Some work on the changes suffered by the hemicelluloses has already been carried out in the United States. It was mentioned that Peterson, Fred, and Verhulst<sup>4</sup> found that 15 to 20 per cent. of the pentosans of the plant material was destroyed during the fermentation, probably, as they suggest, through microbiological activity. In a further publication Fred, Peterson, and Anderson<sup>18</sup> report that *Bacterium lactipentoaceticum* plays an important part in the ensilage of maize by increasing the amount of alcohol and volatile acids formed. Apparently this organism has an inhibitory influence on other lactic acid-producing bacteria, since the American authors find that the total yield of lactic acid is diminished in its presence.

The question of the reactions of sour silage has been studied by several investigators. Jones and Gibbard state that the hydrogen ion concentration of the sweet clover which they used in their experiments was 6.7. On the seventh day of the fermentation the acidity had increased to pH 4.9 and on the fourteenth day to pH 4.6. The highest acidity reached in their experiments was equal to a pH of 4.4, a figure which is unlikely to be exceeded in any silage fermentation. Edin and Sandberg<sup>7</sup> found that the total acidity of their silage samples varied between 0.93 and 2.34 per cent., and that the lactic acid concentration varied between 0 and 1.90 per cent. Swanson and Tague<sup>6</sup> recorded an acidity of between 2 and 3 per cent., calculated as lactic acid. In good silage Edin and Sandberg found no butyric acid, while the lactic acid concentration did not fall below 0.78 per cent. On an average the ratio between the concentration of volatile and lactic acid was found by them to be 1:0.88, with a variation between the ratios of 1.0:1.77 and 1.0:0.44. In Swanson and Tague's samples, 25 per cent. of the total acids consisted of volatile acids where the raw fibre content of the plant material was high. The bulk of these volatile acids, according to most

statements, appears to be acetic acid, which is sometimes (Samarani <sup>12</sup>) regarded as an oxidation product of the alcohol present, but is more likely to be the result of the activity of pentose-fermenting bacteria (Fred, Peterson, and Devonport <sup>13</sup>). The presence in silage of propionic and butyric acids has been reported by Sherman <sup>16</sup>. He and most other investigators attribute the production of lactic acid to members of the *Bact. bulgaricum* group, bacteria which ferment hexoses only and must, therefore, break down the bulk of the soluble hexoses present in the plant tissues in order to account for the comparatively high percentages of lactic acid in sour silage. But since appreciable quantities of alcohols are also claimed to be derived from this source, it is probable that at least part of the lactic acid may be formed from pentosans, a view which is supported by Fred, Peterson, and Anderson's <sup>18</sup> investigations.

The evolution of carbon dioxide is a marked feature of all silage fermentations. The production is associated by Lamb with enzymatic activity of both plant tissues and micro-organisms, and very likely involves a destruction of hemicelluloses.

Alcohol production, which Esten and Mason <sup>14</sup> as well as Lamb <sup>8</sup> attribute to the activity of yeasts, is more likely to be the result of the action of bacteria, and may be due to the activity of pentose-fermenting bacteria related to *Bac. aceto-ethylicus*, Northrop <sup>10</sup>.

The evolution of heat, which is now ascribed to the activity of micro-organisms, is probably not characteristic for one single type, though the members of the aerobic soil bacilli, *Bac. subtilis* and *Bac. mycoides*, which have been reported as present in silage by Emmerling <sup>11</sup>, may be active in this respect. If so, hemicelluloses would also be involved in this reaction.

Of the changes suffered by *cellulose* and *pectin* during ensilage nothing is known. Pectin decomposers in the form of *Clostridium* and *Granulobacter* have been reported by Emmerling <sup>11</sup>. *Bac. subtilis* and *Bac. mycoides* may also be active in the disposal of this substance, though this is unlikely after anaerobic conditions have become firmly established.

From this very incomplete outline of the microbiology of silage it will be seen that fungi take little, if any, part in the ensilage process. Their activity is probably restricted under normal conditions to the surface of the silage heap where oxygen is available.

Of the activity of actinomycetes, which are undoubtedly present in the raw material and in the silage (Schütze<sup>20</sup>), nothing has yet been established. A study of silage on the lines suggested by Henneberg<sup>21</sup> for the investigation of the intestinal microflora would be of great value in throwing light on the importance of both fungi and actinomycetes in the normal progress of the ensilage process.

It remains to point out that Griffiths<sup>2</sup> isolated from silage, and named two thermophilic bacteria, *Bac. thermicus* and *Bac. valericus*, which he considered capable of converting plant tissues into sweet silage. Both types develop at temperatures as high as 60° C., which may be established by packing the plant material sufficiently loosely to admit oxygen to the heap. The description given by Griffiths of these micro-organisms is not detailed enough to show whether *Bac. thermicus* and *Bac. valericus* decompose pectin, hemicelluloses, or perhaps even cellulose, and both these and other thermophilic types which may very likely be present in silage require renewed investigation.

Since this chapter was begun an interesting account of the principal changes which take place in the preparation of silage has been published by Peterson, Hastings, and Fred<sup>22</sup>. Their experiments were conducted in a specially built silo filled with high-grade maize stalks. It was found that the oxygen present disappeared completely during the first five hours after the filling of the silo, and that the carbon dioxide increased rapidly during the first 48 hours, when it amounted to 65 per cent. of the total gases evolved. No hydrogen or hydrocarbons could be detected. During the first fifteen days of fermentation the temperature of the material rose 7° C. near the bottom and 20° C. near the top. From this maximum it fell, but even after 70 days it was still above normal. During the first 48 hours the number of bacteria

increased rapidly and with them the methyl and ethyl alcohol content and the lactic acid concentration. Of the types of bacteria found, the pentose-fermenting lactic acid-producing types were in a majority. The yeast types, on the other hand, diminished rapidly in numbers after the first day.

A chemical analysis showed that 10 per cent. of the total dry matter, 25 per cent. of the pentosans, and 25 per cent. of the starch present had disappeared at the end of four months.

Of particular interest is the statement that sterilized maize stalks inoculated with *Bact. lactipentoaceticum* yielded a silage similar in composition to normal silage.

**The spontaneous heating of hay.**—When hay or other plant material is loosely stacked before it has become dry, it shows a tendency to heat or sweat, a phenomenon which in extreme cases may result in its spontaneous ignition. As in the case of ensilaging, the reactions involved have been ascribed to microbiological, plant physiological, and chemical agencies, but in spite of statements to the contrary available evidence undoubtedly favours the explanation that micro-organisms are responsible for most of the earlier stages of the reactions. Mische<sup>23</sup>, who undertook a comprehensive study of the phenomenon, came to this conclusion, and Haldane and Makgill<sup>24</sup> in a later publication confirm this view after due consideration of the evidence brought forward by Boekhout and de Vries<sup>25</sup> in favour of a chemical explanation, and of Tschirch's<sup>26</sup> suggestions, attributing the heating to the activity of oxydases and reductases secreted by the plant tissues.

Mische made his observations partly on a large hayrick of specially damp hay constructed for the purpose, and partly on small samples of hay contained in suitable vessels so designed as to prevent loss of heat by radiation. In the rick Mische found that the temperature started to rise shortly after the construction of the rick, and on the third day it had reached 57.5° C. at a depth of 185 cms. Nearer the surface, to a depth of about 20 cms., only slight temperature changes occurred, and here the maximum never exceeded 32° C., though the centre of the rick reached 68.5° C. on the fifth day, and remained at that level until the seventh day. After that time

the temperature dropped, but did not fall below 50° C. until a month after the construction of the rick. Generally speaking the temperature increased steadily towards the centre of the heap, provided its moisture content was uniform. Where the material varied in this respect a number of centres of maximum temperature occurred in the interior of the rick, associated with the damper layer of plant material. This observation had previously been made by Dügge<sup>27</sup>.

The chemical changes which the plant material suffered during heating in Miehe's experiments comprised a gradual loss of moisture, the water escaping as visible vapour. These losses sometimes proceeded far enough to reduce the moisture content of the heated plant material to 60 per cent. of that usually held by dry hay.

Of the carbohydrates present in the hay the pentosans lost about 40 per cent. of their original weight. The total nitrogen was reduced, in some cases to the extent of 50 per cent., though the protein present was little affected, an observation which does not seem to agree with what occurs in the preparation of silage. The breakdown of the carbohydrates resulted in the formation of butyric, acetic, and probably formic and lactic acids, and in the evolution of carbon dioxide. Methane and hydrogen were not observed in measurable quantities.

From the heated hay Miehe isolated several micro-organisms with which he was able to reproduce spontaneous heating in samples of sterilized hay. The two most important types were named *Bact. coli* forma *foenicola* and *Bac. calfactor*, the former causing the preliminary heating during which the temperature of the hay rises to 40° C. At 49° C. *Bac. calfactor*, which does not grow at 30° C., developed rapidly and by its activity increased the temperature to between 70° and 75° C.

The biochemical reactions of *Bact. coli* forma *foenicola* and of *Bac. calfactor*, and particularly their action on pectin, hemicelluloses, and cellulose, still remain to be studied, Miehe apparently having limited his investigations to a determination of their participation in the production of heat. Both of these bacteria are normally present on hay, and may be isolated

from this material; *Bac. calfactor*, by incubating a hay infusion at 60° C.

In addition to these forms Miehé isolated from spontaneously heated hay a thermophilic actinomycete related to *Actinomyces monosporus*, Schütze<sup>20</sup>, but forming white instead of green spores. Fungi were represented by an *Oidium* species, *Thermoidium sulphureum* (Miehé<sup>28</sup>), two *Mucor* species, *Mucor pusillus* and *Mucor corymbifer*, an *Aspergillus*, *Aspergillus fumigatus*, and a higher ascomycete, *Thermoascus aurantiacus* (Miehé<sup>23</sup>). In no case were the biochemical reactions of these eumycetes investigated. It is probable, however, that they take part in the decomposition of either pectin or hemicelluloses, and perhaps, in the case of some of them, in the destruction of the cellulose of the plant material.

It is interesting to note that *Bac. calfactor*, though developing at temperatures up to 74° C., does not remain alive either as spores or as vegetative cells in the interior of a hayrick which has retained a temperature of 65° C. for a month. Not even the spores, therefore, of this thermophilic micro-organism can withstand for any prolonged time the temperature at which protein usually coagulates.

The actual ignition of a heated hayrick is not the work of micro-organisms, but is due, as Miehé and Haldane and Makgill have observed, to the rapid absorption of oxygen by the hay after its prolonged heating and consequent drying. It should be emphasized also that Miehé does not deny the participation of plant enzymes in the early stages of the heating of a hayrick or other accumulations of living plant cells. But this agency, according to him, does not increase the temperature above 40° C.

In discussing the information available on the microbiology of ensilage it was pointed out that much work has still to be done before the reactions involved can be regarded as understood. The same may be said of the spontaneous combustion of hay, an analogous process which occurs partly under aerobic conditions instead of the more or less strictly anaerobic conditions of the silage fermentation.

The elucidation of these processes is by no means as unim-

portant from a practical point of view as might appear at first sight, since the spontaneous heating of accumulated plant material is frequently utilized industrially. The fermentation, for instance, of tobacco, of the cacao bean, and of the coffee bean are processes of a similar nature, the economic importance of which need not be stressed.

**The fermentation of tobacco.**—In the process of tobacco fermentation the dried (cured) leaves are placed in heaps large enough to prevent a rapid loss of heat by radiation. A suitable size for a heap is from 60 to 120 cms. square by 120 to 180 cms. high (Smitz <sup>29</sup>). The moisture content of the heap should reach 20 to 25 per cent. and an excessive drying during curing, which has reduced the moisture content below this figure, may therefore necessitate spraying the heap with water.

A properly constructed heap will show a rise of temperature to 48° C. or even 70° C. in two to four days. This rise indicates that the fermentation has set in. When the optimum temperature has been reached, a point which varies with the type of tobacco required, a further rise of temperature must be prevented. This is done, after three to four days' fermentation, by opening up the heap and rebuilding it in such a way that the leaves previously on the surface are moved to the centre and the centre leaves to the top. The repacking has usually to be repeated from five to ten times at intervals of seven to eight days.

As a result of the fermentation the leaves become darker in colour and acquire the desired flavour through changes involving the pentosans (Boekhout and de Vries <sup>30</sup>), the gums, the starch, the glucosides, the fats and the proteins (Oosthuizen and Shedd <sup>31</sup>). The total loss of dry matter, according to Oosthuizen and Shedd <sup>31</sup>, amounts to from 4 to 5 per cent. The same authors state that gases, the nature of which is given by Boekhout and de Vries <sup>30</sup> as carbon dioxide, are given off during fermentation.

On the above facts most investigators agree, but beyond this point the views of the various authorities are difficult to reconcile. One school, represented in the more recent literature

by Vernhout<sup>32</sup> and by Koning<sup>33, 34</sup>, regards micro-organisms as chiefly responsible for the changes. Another, represented among others by Loew<sup>35</sup> and by Oosthuizen and Shedd<sup>31</sup>, attributes the fermentation to enzymes contained in and produced by the tobacco plant, while a third school, Jensen<sup>36</sup>, Cohn and Jensen<sup>37</sup>, and Boekhout and de Vries<sup>30</sup>, denies the activity of either micro-organisms or enzymes and regards inorganic catalysts (Boekhout and de Vries), such as the iron contained in the leaves, as the chief agent of the fermentation, accelerating not only the oxidation of the carbohydrates, but also the saponification of the glucosides and the fats, and the proteolysis of the proteins. The chief argument of this last school is that the changes in the tobacco leaf can take place in the presence of antiseptics such as chloroform, and at temperatures as high as 100° C., under conditions in which neither micro-organisms nor enzymes would be capable of acting. Though undoubtedly serious, these objections cannot invalidate the possibility of the participation of either micro-organisms or plant enzymes under natural conditions, where such extreme conditions do not exist, and where in fact the high temperatures are distinctly dangerous.

The school, particularly Loew<sup>35</sup>, which attributes all the changes occurring during fermentation to plant enzymes, advances as an objection against the participation of micro-organisms the fact that the moisture content of the fermenting heap is too low for growth, and that the tobacco leaf is an unsuitable nutrient medium for the development of micro-organisms. The first of these objections no longer holds good (see Chapter XI), and the second is hardly more convincing, since a luxuriant growth of a variety of micro-organisms may be obtained on tobacco which has been stored damp.

Though it must be admitted that micro-organisms can develop in fermenting tobacco under natural conditions—and the 'thousands of colonies' obtained by Koning<sup>34</sup> from a small piece of leaf confirms this—the authorities who advocate the importance of micro-organisms have unfortunately not yet advanced convincing evidence to show that the types isolated by them, types which resemble those bacteria isolated by



Miehe<sup>23</sup> from spontaneously heated hay, are essential for the changes which occur in the leaves during fermentation.

The whole question of the fermentation of tobacco, and of the equally important problem of the destruction of fermented tobacco in storage, undoubtedly requires renewed investigation.

**The fermentation of the cacao and the coffee bean.—**

Whether the fermentation to which the cacao and the coffee bean are subjected after gathering involves the decomposition of cellulose, hemicelluloses, or pectin is another question which requires elucidation. That the changes wrought in these cases, or at least in the case of the cacao bean, which has been most carefully studied, are essential (Knapp<sup>38</sup>), and are associated with the activity of micro-organisms (Preyer<sup>39</sup> and Nicholls<sup>40</sup>), is beyond doubt; though opinions are not lacking (Loew<sup>41</sup>, Schulte im Hofe<sup>42</sup>, Fickendey<sup>43</sup> and Brill<sup>44</sup>) that the responsibility rests solely with the activity of plant enzymes contained in the pulp surrounding the fresh beans.

In the case of cacao—coffee has as yet hardly been studied—the fresh beans embedded in a sweet pulp are placed in fermentation boxes immediately after removal from the pods and are there allowed to undergo a fermentation process, which causes the temperature of the mass to rise to 45° C. or even 50° C. As in the case of fermenting tobacco leaves, an excessive production of heat is counteracted by frequent transfer of the heated mass to fresh boxes. As a result of the fermentation the embryo of the bean is killed, the pulp rendered easily removable by washing, and the tannic substances present oxidized and converted into insoluble compounds. At the same time the characteristic flavour of the cacao bean is developed or enhanced. The fermentation takes from one to nine days to complete according to the type of bean used.

Since Preyer's investigations the yeasts found, such as *Saccharomyces theobromae*, have been regarded as the responsible micro-organisms, perhaps in conjunction with acetic acid-producing bacteria (Nicholls). Other forms, however, are undoubtedly associated with them, since the liquid which drains from the fermenting mass has been shown to contain both lactic and butyric acids, in addition to acetic acid and ethyl

alcohol. Nor is it likely, as Knapp<sup>45</sup> points out, that the yeast forms present constitute the chief microflora, at least in the later stages of fermentation when the temperature rises much beyond that usually suitable for these eumycetes.

Just as the tobacco fermentation shows analogies to the spontaneous combustion of hay, so do the cacao fermentation and perhaps the coffee fermentation resemble the silage fermentation, and much light could undoubtedly be thrown on both of these processes if they were considered from that point of view.

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## CHAPTER X

### PEAT AND COAL

It is now universally conceded that peat, lignite, and all types of coal owe their origin to the accumulation and decay of vegetable debris and that these fuels represent so many stages in the progressive carbonization of plant tissues.

In an interesting treatise by White and Thiessen<sup>1</sup> on the origin of coal, Thiessen summarizes his microscopical observations in the following sentences: 'All coal was laid down in beds analogous to the peat beds of to-day. All kinds of plants, especially such species as were adapted to the particular region where the deposit was located in whole or in part, went into the deposit. . . . At the death of the plants, governed by conditions imposed in the bog, a partial decomposition, maceration, elimination, and chemical reduction began, brought about by various agencies. . . .'

If this statement may be taken as essentially correct, and to-day nobody seriously opposes it, an account of the part played by micro-organisms in the production of peat would be a description also of their participation in the formation of lignite and coal and possibly also in the formation of mineral oil, which some authorities consider to be derived from vegetable matter.

An investigation of peat from this point of view has unfortunately not yet been undertaken, and those interested in the microbiological aspect of the formation of coal and other solid and liquid fuels of past ages have consequently been compelled to base their deductions either on direct observation of signs of microbiological activity in the fuels, or on a comparison with microbiological processes occurring in already studied types of decay of vegetable debris. It is from the latter point of view that an attempt will be made in the following pages to throw light on the question of the partici-

pation of micro-organisms in the formation of peat and consequently of lignite, coal, and oil.

Before doing so an outline will be given of the conditions under which vegetable debris accumulates to form peat and coal. Readers interested in a detailed study of this subject are referred to the standard text-books, and to the lucid expositions of White and Thiessen<sup>1</sup> and of Stopes and Wheeler<sup>2</sup>.

The plant debris, according to Stopes and Wheeler, may have accumulated:

*In sea water*, and been derived from:

(1) Drifted land material which had travelled far and therefore had become waterlogged. The material in this case might have consisted of:

- (a) tree trunks and logs;
- (b) floating islands of various plants growing entangled.

(2) From higher algae, seaweed, forming a shore accumulation.

*In brackish water*, and been derived from:

(1) Material growing *in situ*, for instance the debris of coastal forests and mangrove-like swamps;

(2) material growing in low-lying swamps and bogs;

(3) partly from such material in addition to material drifted into the swamps from shorter distances;

(4) the material described under 1, 2, and 3 mingled in any proportions.

*In fresh water*:

(1) In undisturbed lakes:

(a) from the gross debris drifted in from neighbouring forests;

(b) from the plankton of the lake;

(c) from the plankton mixed with spores, pollen grains, and finer debris of higher plants;

(d) from plants growing *in situ* such as reeds, &c.

(e) from a, b, and c mixed or layered in any proportions.

(2) In estuaries, river bends, and deltas:

(a) from drifted material, which may have travelled far, principally from wood;

(b) from drifted material near at hand, including leaves, &c., in addition to wood;

(c) from floating islands;

(d) from swamp and bog plants growing *in situ*;

- (c) from material as under *a*, *b*, and *d*, mingled in any proportions.
- (3) In swamps formed over large areas interspersed with lakes, from *in situ* and drifted material mixed.

*On land:*

- (1) from material growing on highland moors, including mosses;
- (2) from material on moors, mingled with forest debris;
- (3) from material of swamps;
- (4) from the accumulation of dry forest floors.

The major deposits of earlier epochs were formed in gradually subsiding brackish and freshwater swamps, possibly also in undisturbed lakes, in estuaries, river bends, and deltas, that is, under conditions by which the debris rapidly became waterlogged, though not necessarily deeply submerged.

The decomposition of the great thickness of peat necessary for the production of a thick bed of coal, which requires from 10 to 20 ft. of peat to produce one foot of high-grade bituminous coal, could hardly have taken place, White<sup>1</sup> states, except under such close adjustment of the rate of subsidence to rate of peat accumulation as would maintain a depth of water within limits which permitted the growth of the peat-forming vegetation for an exceedingly long time. Too rapid subsidence would have flooded the swamps so deeply as to kill the principal peat-forming vegetation and allow the invasion of sediment-forming water. On the other hand, had the subsidence of the region been too slow, the surface of the peat might have reached the upper limit of its formation and entered the zone of increasing exposure (aerobic conditions) in which the organic matter would become reduced to a humus soil or possibly have been completely destroyed. Irregular temporary reductions or withdrawals of the water, either seasonally or perhaps less frequently, White suggests, might have been causally related to the ordinary type of lamination of much of the existing coal, and might have been responsible for the admixture to the latter of fragments of mineral charcoal ('mother of coal' or 'fusain').

The climate at the time of the accumulation of the larger deposits of coal has been variously described as typically

tropical (Potonié<sup>3</sup>), as sub-tropical (Dannenberg<sup>4</sup>), to mild (White and Thiessen<sup>1</sup>), and possibly at times rather cold (Kukuk<sup>5</sup>), not unlike that existing to-day in southern Chili and in the Chatham Islands. That it must have been exceedingly humid and uniform throughout the year is a fact on which all authorities agree and which may be deduced from the morphological structure of the residual vegetation of the old deposits, which show no sign of annual growth rings. During the deposit of younger accumulations, giving rise to peat and lignite formation, climatic conditions were probably very similar to those existing to-day in the temperate zones, since the remains of vegetation found in these deposits are identical with those found to-day in colder climates.

The plant material which became deposited appears to have consisted partly of unlignified parenchymatous tissues represented chiefly by mosses, herbaceous plants, and pith, partly of lignified structures including leaves, twigs, branches, and even trunks, and partly of suberized tissues such as bark and spores with their wax and resin content. The relative proportion of unlignified to lignified structures originally entering the peat bog must have varied considerably, the latter undoubtedly preponderating in many cases.

An impression may be gained of the mode of accumulation of the debris from White's account of the progress of peat formation in the large peat deposit of the so-called 'Dismal Swamp' of Virginia and North Carolina, and in the inland swamp of eastern Sumatra explored by Koorders and Potonié<sup>3</sup>. These deposits, according to White, are the most representative existing samples of that type of swamp in which vegetation became accumulated to form coal, though the climatic conditions in the case of the Sumatra swamp may be more tropical than those prevailing when the ancient peat bogs were formed.

Before attempts at drainage were begun in the great Dismal Swamp, it was covered permanently with a shallow layer of water over the greater part of its expanse. So far as it has been explored it consists of a dark brown, in places reddish-brown, peat reaching 240 to 360 cms. down. The peat consists in large measure of small plant fragments including

cuticles, pieces of bark, seeds, twigs, roots, and many branches and logs of considerable size, lying at various levels. In fact, so abundant are the logs—and apparently so little decomposed—that in parts of the swamp an iron rod cannot be forced down to a depth of 180 cms. The flora of the swamp is largely angiospermous, though the bald cypress and the white cedar (*Chamaecyperis thujoides*) are scattered over certain areas. Sphagnum moss occurs in places and its debris, together with resin, spores, and pollen, are undoubtedly present in the peat.

The peat swamp of eastern Sumatra is found in an inland plain far from the coast. It covers more than 80,000 hectares. In it there is being gradually deposited a high-grade peat reaching a depth of 9 metres and having only 6.39 per cent. of ash in the dry fuel. The peat includes many remains of phanerogamous plants, including pollen, and also the spores of cryptogams. Debris of mosses, on the other hand, is rare. It is covered by about 60 cms. of tea-coloured fresh water. By far the greatest part of the peat-forming substance is supplied by a dense forest of mixed evergreen trees which form a canopy over the swamp and whose leaves, dead twigs, and trunks fall into the water. The trees stand so close together that their roots form a matted tangle at the surface of the peat. Comparatively few herbaceous plants are present.

The rate of formation of peat in this swamp is thought by White to be considerably faster than in bogs of colder climates. Normally some 10 years are required to produce 30 cms. of young peat, but continued decomposition will be responsible for a decrease, and at a depth of 4.5 to 6 metres this amount of accumulated plant debris may have been reduced to a layer of only 2.8 cms. At that rate 30 cms. of peat, at a depth of 45 cms., would take about one hundred years to form.

From the descriptions given it will be seen that peat was and is formed under conditions which favour the development of micro-organisms, at least so far as temperature and moisture conditions are concerned. The fact that during peat formation the vegetable debris rapidly becomes waterlogged in stagnant water in which readily oxidizable plant material is dissolved indicates that any decomposition occurring in the debris must



take place under anacrobic conditions or at least in the presence of a limited supply of oxygen. This is important, since the development of fungi, to which Fischer and Schrader<sup>6</sup> attach so much importance in their theory of the formation of coal, is greatly restricted under such conditions.

Given such comparatively favourable conditions for their growth, it is not surprising that for many years micro-organisms have been associated with the formation of peat and coal. The evidence on which this association is based is very slender, however, and has been derived solely from the microscopic examination of coal preparations. Investigations of the microbiological processes still occurring during peat formation do not seem to have been seriously undertaken, though they would have been infinitely more convincing than direct observations of coal sections, particularly if made to include a study of the presence and distribution of cellulose- and lignin-decomposing types.

The first statement that micro-organisms were associated with the decay of plant material in the formation of coal dates from 1879, when van Tieghem<sup>7</sup> reported that he had observed unmistakable signs of the presence of *Bac. amylobacter* in sections of coal. At that time *Bac. amylobacter* was regarded as the cellulose-decomposing organism *par excellence*, and van Tieghem's statement therefore was of particular interest, since it definitely associated the formation of coal with a microbiological decomposition of cellulose. Some years later Renault<sup>8, 9, 10, 11, 12, 13</sup> gave an account of his examination of coal sections, and described a number of micro-organisms which he had observed in these preparations, and which he connected causally with the formation of coal. Later investigations, among them those of Thiessen<sup>1</sup>, have confirmed the presence of micro-organisms in coal, chiefly in the form of fungus spores. And Grüss, quoted by Lessing<sup>14</sup>, reports having observed the mycelium of fungi in a specimen of coal-like formation occurring in the Devonian limestone of Spitzbergen. Living micro-organisms have been reported as present in peat by White and Thiessen at a depth of 835 cms. below the surface of the peat bog, a remarkable statement, since

such organisms must either have been continually active for some 2,000 years, the time required to form this layer of peat, or if inactive must have survived an amazingly long period in the peat in which they were found. It is most regrettable that these micro-organisms were not subjected to a detailed investigation, particularly with a view to determining their ability to live in peat under anaerobic conditions and their action on cellulose and lignin.

Fremy's<sup>15</sup> views did far more than these scattered observations to spread the belief in the participation of micro-organisms in the formation of peat and coal. Fremy suggested that these fuels had undergone a *fermentation tourbeuse* by which the accumulated plant debris had become converted into an amorphous compound, in which individual plant tissues which had escaped the action of the micro-organisms had become embedded. The amorphous compound was undoubtedly regarded by Fremy as related to, or even identical with, the humic substances, that is, the plastic compound of a well-rotted farmyard manure. According to Fremy it had been derived both from the cellulose and from the lignocellulose of the plant material undergoing the *fermentation tourbeuse*.

Further light is thrown on this fermentation by White and Thiessen, who describe it as the biochemical stage of peat and coal formation. During this stage, which is stated possibly to be due principally to the activity of fungi at first, but in which bacteria certainly play the chief role later, the accumulated vegetation becomes more or less disintegrated and decomposed. The operation varies constantly both in phase and extent, even in the same bed or locality, according to the ever varying conditions of deposition and the nature of the debris contributed. The process, White says, ends with the cessation of anaerobic microbiological action as a result of exhaustion of the necessary supply of oxygen [*sic*], or the development of destructive toxins. The microbiological action may, in White's view, result in a partial decomposition of the vegetable debris, whereby products such as the xyloid lignites and the woody or fibrous peats are formed. In early geological times it may have gone farther and yielded densely laminated coal. In

extreme cases it may have obliterated almost completely all the plant structure and thus given rise to the so-called 'amorphous' coals and peats.

How far and for how long this microbiological decomposition process may continue in peat is not clearly stated. White expresses the view that its duration will vary with the rate of growth of the peat, with the toxicity of the decomposition products, with the porosity of the superficial layers of young peat, and with temperature, water composition, and other factors. He says also that there is reason to believe that the anaerobic action may continue at considerable depths in the bog, even for some time after the peat deposit has been covered by sediment. He considers it probable that it is still in progress in most of the peat studied. Though believing in an extensive participation of microbiological activity, White does not agree with the theory that differences in the type of micro-organisms active during the formation of the peat can be made responsible for differences in the nature of the final coal.

In Thiessen's opinion the microbiological stage will remove the most labile compounds of the plant tissues first, proceeding to the more resistant as conditions require, and leave behind finally only the most resistant debris, including resins and waxes. Though cellulose cannot be regarded as a material particularly resistant to microbiological destruction, Thiessen emphasizes that the peat still contains a large percentage of this substance.

The participation of micro-organisms in the formation of peat, and consequently of coal, has recently been somewhat differently described by Fischer and Schrader<sup>9</sup>. These authors consider it unlikely that cellulose, except in insignificant quantities, can have escaped the action of the micro-organisms which took part in the decay of the accumulated vegetation, and they regard peat, lignite, and coal as having been formed essentially from the lignin content of the decayed plant tissues. They evidently regard fungi as the chief types responsible for the removal of cellulose, since they stress the importance of Bray and Andrews's<sup>16</sup> investigations on the destruction of wood by these organisms, and quote Darwin's<sup>17</sup>

remarks on the decay of tree trunks in the primeval forests of South America in support of their theory. The importance of fungi in the formation of peat, and consequently of coal, has also been emphasized by Wehmer<sup>18</sup>, in spite of the fact that the moisture content of peat bogs is far in excess of that suitable for the development of these micro-organisms.

From the above outline it will be seen that it is generally agreed that micro-organisms are present during the decay of the accumulated plant tissues which leads to the formation of peat, and that they take a very conspicuous part in its progress. It should not be overlooked, however, that, apart from a passing reference by Stoklasa<sup>19</sup>, this assumption is based on tentative considerations and not on proved facts.

In the course of time the views held on the chemical composition of that part of the decaying vegetable debris which went to form peat and coal have crystallized into two distinct theories. One of these, supported by White and Thiessen, and Stopes and Wheeler, sees a raw material for peat and coal formation in both cellulose and lignin, while the other, and newer, theory advanced by Fischer and Schrader disregards the cellulose and makes lignin alone responsible.

At first sight there is a good deal to be said for Fischer and Schrader's standpoint, even from the point of view of the bacteriologist. Thus, it is a frequently established fact that the microbiological decay of pure cellulose does not give rise to the formation of natural humic substances. It is noteworthy also that many fungi (though by no means all), when attacking wood and wooden structures, decompose the cellulose more or less completely, leaving the lignin almost untouched. But to gain a true impression of the actual occurrences during the formation of peat—conceding for a moment that micro-organisms do take part in it—it is not sufficient to contemplate what may happen under exceptional circumstances. On the contrary, the subject must be considered from the point of view of the progress of the decay as it usually occurs, that is under waterlogged conditions and in the more or less complete absence of oxygen.

It is not difficult to contemplate conditions under which

vegetable material will decay under waterlogged and more or less anaerobic conditions. The preparation of silage offers a very good illustration of such conditions, though the microbiological decay in this case undoubtedly proceeds under more favourable conditions than during the formation of peat, especially as regards range of temperature and the presence of a more readily decomposable plant material. The main features of silage preparation have already been outlined, and it has been pointed out that the fully ripened silage, in which all microbiological activity has ceased, still contains large quantities of cellulose, and even pentoses, in addition to lignin and humic substances. But if micro-organisms are unable to complete the destruction of cellulose during the silage fermentation, are they likely to do so during the decay of the heterogeneous material which is deposited in a peat bog? Is it possible, it may even be asked, that their activity can carry the decay as far as in the silage fermentation under the comparatively unfavourable conditions existing in a peat bog, where both the peat itself and the water in which the vegetable debris becomes submerged have been found to check the development of micro-organisms?

The latter question cannot of course be answered until the microbiology of the active peat bog has been investigated. But assuming that the decay in the peat bog does proceed under waterlogged and more or less anaerobic conditions, there is every justification for asserting that, in the best of cases, microbiological activity must cease in the peat before the cellulose and the hemicelluloses of the submerged plant material have been even approximately eliminated. The complete disintegration of the plant material and the progressive elimination of the cellulose, which Fischer and Schrader have undoubtedly shown to occur, must therefore be performed by other agencies than micro-organisms, which it would be beyond the scope of this volume to discuss.

Some evidence for this conception of the formation of peat and coal has been brought forward in a recent paper by Thaysen, Bakes, and Bunker<sup>20</sup>. It is suggested in this publica-

tion that microbiological activity ceases in the upper layers of the peat bog before the bulk of the cellulose has disappeared, and that the subsequent destruction of the cellulose proceeds on lines similar to those active in the production of carbohydrate humus from linen cloth through ageing. It is shown that the humus compounds of a normal peat bog, after chlorination by the method recommended by Eller and his collaborators<sup>21</sup>, can be split up into two substances, one identical with the humus compound obtainable from quinones and lignin, and the other with the composition of the artificial humus compound obtainable from sugars and other carbohydrates by the action of acids. The latter type of humus was also obtained by these workers from samples of Egyptian mummy cloth made of pure undyed linen which had decayed in the absence of micro-organisms.

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## CHAPTER XI

### CELLULOSE FIBRES AND FABRICS

IN an earlier chapter the participation of micro-organisms in the retting of fibres was reviewed and the value of their application in this direction discussed. An account will now be given of the damage caused by micro-organisms in the textile industries, starting with the 'mildewing' of fibres and fabrics.

By mildewing is understood the growth of fungi, and possibly of bacteria (Osborn<sup>1</sup>), on fibres and fabrics, which results in the discoloration, and frequently in the disintegration of the material on which it occurs. The patches seen on mildewed textiles are often vividly coloured, either black, green, yellow, or pink, depending on the colour of the spores or the mycelium of the responsible micro-organisms, on the nature of the pigments secreted by them, or on the chemical changes produced in the dyes with which the mildewed fabrics may have been treated.

The earliest investigations on the mildewing of cotton goods are recorded in publications by Thomson<sup>2</sup> and by Davis, Dreyfus, and Holland<sup>3</sup> dating from 1877 and 1880 respectively. Though the damage caused by the development of these fungi was then already realized to be serious, a systematic investigation of mildewing was not undertaken and could not perhaps have been undertaken at the time owing to lack of technical facilities. It is regrettable, however, that a thorough study of the problem should have been deferred until quite recently, and that in consequence even the most recent text-books on textiles, when dealing with the subject of mildewing, have had to rely for their information on the frequently irrelevant and certainly quite insufficient data supplied by the authors referred to above.

The first attempt to study mildew in its various aspects was made by Bright, Morris, and Summers<sup>4</sup> as recently as in 1924, and so far theirs is the most comprehensive account of the action of fungi on cotton and cotton fabrics. Reference will frequently be made to their treatise in the following pages.

The question of the origin of the infection was studied in some detail by Bright, Morris, and Summers, who came to the conclusion that mildewing might in some cases be the result of a contamination of the raw cotton, while in others, where the fabrics had undergone such drastic processes as bleaching or mercerization, the original microflora of the cotton hairs would have been destroyed. Here a subsequent infection would therefore be responsible for the mildewing. As fungi likely to cause mildewing in such cases, Bright, Morris, and Summers mention *Penicillium*, *Aspergillus*, and *Rhizopus*, which they say may be frequently met with in cotton warehouses and mills. That *Penicillium* and *Aspergillus* should be regarded as typical mildewing fungi is denied by Guéguen<sup>5</sup>, who holds the view that the infection is invariably to be traced to the cotton plant. Guéguen's view does not harmonize, however, with the frequently reported occurrence of *Penicillium* and *Aspergillus* growths on mildewed fabrics, nor with the undoubted capacity of many species of these genera to decompose cellulose. It is probably justifiable to conclude with Bright, Morris, and Summers that the infection leading to mildewing may in some cases arise in the raw material, and in others be the result of a subsequent contamination. In either case certain conditions as to moisture content, temperature, and food materials must be established before the development of the contaminating micro-organisms can take place.

The minimum moisture content which will enable micro-organisms to develop on raw cotton was estimated by Fleming and Thaysen<sup>6</sup> to be about 9 per cent. Armstead and Harland<sup>7</sup>, who paid particular attention to the moisture requirements of fungi, found that an *Aspergillus* with which they worked could develop slowly on cotton containing only 7.8 per cent.



of water, a figure which, as Osborn points out, may frequently be reached and even exceeded in the Lancashire cotton mills. On the whole, species of *Aspergillus* appear from Reiners's<sup>8</sup> investigation to require less moisture for growth than other fungi, a fact which undoubtedly contributes to the frequent occurrence of this type of fungus on mildewed fibres and fabrics.

As regards temperature, it is a well-known fact that fungi can develop at a few degrees above freezing-point, and rapid growth occurs between 15 and 30° C., temperatures prevalent in most places where fibres and fabrics are stored or handled.

Hardly more difficult to satisfy are the food requirements of fungi. It has been shown by Sée<sup>9</sup> that fungi will develop on paper consisting principally of cellulose and containing little nitrogen, and certainly only mere traces of organic nitrogen. This can only be due to their faculty of being able to satisfy their nitrogen requirements from ammonia and other inorganic nitrogen compounds present in the air, or to their ability to fix atmospheric nitrogen, a property which Froehlich<sup>10</sup> found to be common among lower fungi such as *Alternaria*, *Penicillium*, and *Cladosporium*, which could be grown permanently in a medium containing no chemically bound nitrogen. The quantities of atmospheric nitrogen fixed by these fungi, when calculated on the carbohydrate decomposed by them, were found by Froehlich to exceed those fixed by such recognized nitrogen-fixing micro-organisms as *Clostridium Pasteurianum*.

It has already been shown that the range of carbohydrates which fungi are able to utilize covers cellulose, hemicelluloses, and pectin. In addition, they utilize the more readily decomposable polysaccharides such as starch and inulin, as well as most di- and monosaccharides. Their food requirements, therefore, are fully provided for in all fibres and fabrics, particularly in those in which starch and similar sizing materials have been incorporated. Even lignified fibres, if not too heavily encrusted, can be utilized by fungi, as will be shown in the following chapter.

Since most of the fungi which are commonly met with on fibres and fabrics produce vividly coloured conidiospores, and in addition often secrete yellow, reddish, or brown pigments, as well as reductases, it is not surprising that the presence of mildew becomes apparent through the discoloration of the material on which it occurs. Only in the later stages, as the growth of the fungi progresses, does the cellulose material become seriously involved and suffer what is technically known as tendering, that is, become brittle and, in extreme cases, converted into a dust. In spite of the pigmentation of the attacked material, it is not usually possible to identify the fungi responsible for the mildewing except by their isolation in the laboratory. Neither the colour of the conidiospores nor the pigment secreted are sufficiently characteristic of any one species to be of use for this purpose. At the best, a certain discoloration may be regarded as an indication of the presence of a given type of fungus.

Though a complete list of all fungi associated with mildewing has not yet been compiled, a good many types have been isolated and described. They are included in the list given below. For a more detailed description of these fungi reference should be made to Chapters V and VI.

As several of the types mentioned do not decompose cellulose, their development on mildewed goods must be due to the presence on the material of substances such as starch, contained in the size, which can be used as food by these fungi. This illustrates one of the dangers of the use of sizes containing starch. These sizes favour the appearance of fungi which otherwise could not have occurred as mildew, except perhaps in cases where the textile has been previously attacked by cellulose-destroying forms and where in consequence readily digestible carbohydrates might have been produced.

Mildewing has frequently been studied microscopically and the impression gained from the earlier accounts is that the threads, and sometimes the individual fibres and hairs of mildewed fabrics, become enveloped during the attack by masses of hyphae, from which conidiophores with pigmented spores arise. The figure given illustrates this (Fig. 10).

TABLE I

Type of fungus.	Material from which isolated.	By whom isolated or recorded.	Character of pigments produced.	Cellulose decomposing power.
1. <i>Stachybotrys</i>	Cotton fabrics	Davis, Dreyfus & Holland <sup>5</sup>	Greenish to greenish-black	+
2. <i>Aspergillus</i>	Cotton hairs and fabrics. Flax and hemp fibres	Davis, Dreyfus & Holland <sup>5</sup> Behrens <sup>11</sup> Osborn <sup>1</sup> Levine & Veitch <sup>12</sup> Deróo <sup>13</sup> Armstead & Harland <sup>7</sup> Ruschmann <sup>14</sup> Reiners <sup>8</sup> Bright, Morris & Summers <sup>4</sup>	Usually greenish conidio-spores and yellowish pigments	+
3. <i>Penicillium</i> (various species)	Cotton hairs and fabrics. Flax and hemp fibres	Davis, Dreyfus & Holland <sup>5</sup> Osborn <sup>1</sup> Hauman <sup>15</sup> Armstead & Harland <sup>7</sup> Ruschmann <sup>14</sup> Bright, Morris & Summers <sup>4</sup>	Greenish conidio-spores	+
4. <i>Mucor</i> and <i>Rhizopus</i> (various species)	Cotton hairs and fabrics. Flax fibres	Behrens <sup>11</sup> Osborn <sup>1</sup> Levine & Veitch <sup>12</sup> Hauman <sup>15</sup> Ruschmann <sup>14</sup> Reiners <sup>8</sup> Bright, Morris & Summers <sup>4</sup>	Dark brown to black conidio-spores	—
5. <i>Alternaria</i>	Cotton hairs and fabrics. Flax fibres.	Levine & Veitch <sup>12</sup> Bright, Morris & Summers <sup>4</sup>	Blackish conidio-spores	+

TABLE I (continued)

Type of fungus.	Material from which isolated.	By whom isolated or recorded.	Character of pigments produced.	Cellulose decomposing power.
6. <i>Cladosporium</i>	Cotton hairs and fabrics. Flax and hemp fibres	Davis, Dreyfus & Holland <sup>3</sup> Levine & Veitch <sup>12</sup> Hauman <sup>16</sup> Ruschmann <sup>14</sup> Bright, Morris & Summers <sup>4</sup>	Olivegreen to brown, almost black	+
7. <i>Botrytis</i>	Cotton hairs and fabrics. Flax fibres	Behrens <sup>11</sup> Sidebotham <sup>16</sup> Bright, Morris & Summers <sup>4</sup>	Dark olive to brownish conidiospores	+
8. <i>Fusarium</i>	Cotton hairs and fabrics	Osborn <sup>1</sup> Armstead & Harland <sup>7</sup> Bright, Morris & Summers <sup>4</sup>	Pink, violet and cherry-red pigments	+
9. <i>Stysanus</i>	Cotton hairs and fabrics	Osborn <sup>1</sup>	Brown to blackish mycelium	?
10. <i>Chaetomium</i>	Cotton hairs and fabrics	Davis, Dreyfus & Holland <sup>3</sup> Osborn <sup>1</sup> Bright, Morris & Summers <sup>4</sup>	Greenish mycelium	+
11. <i>Macrosporum</i>	Cotton and linen canvases	Broughton Alcock <sup>17</sup> Ramsbottom <sup>18</sup>	Dark brown to black conidiospores	?
12. <i>Stemphylium</i>	Cotton and linen canvases	Broughton Alcock <sup>17</sup> Ramsbottom <sup>18</sup>	Dark brown to blackish conidiospores	+
13. <i>Dematium</i>	Cotton hairs	Bright, Morris & Summers <sup>4</sup>	White to pinkish	—
14. <i>Acrothecium</i>	Cotton hairs	Bright, Morris & Summers <sup>4</sup>	Brownish to black conidiospores	?
15. <i>Oidium</i>	Flax and hemp fibres	Ruschmann <sup>14</sup>	Whitish	—

The presence of large masses of mycelium covering the surface of the cellulose fibres led to the assumption that the attack of the fungi started on the surface of the fibres or hairs and gradually proceeded inwards. This view was in principle adhered to by Fleming and Thaysen<sup>19</sup>. It has been attacked, however, by several authors, chiefly by Denham<sup>20</sup>, who studied the attack of fungi on cotton hairs. Denham finds that the cotton hair is liable to attack in several places both within and on the outside of the hair. These places, in order of vulnerability, are: broken ends of hair, deep cracks reaching the lumen, abrasions, shallow cracks and pits, and the normal surface of the hair or cuticle. Infection by the central canal, the lumen, and not from the surface of the hair is according to Denham by far the most common, because of, as he suggests, the chemotactic action exercised on the hyphae by the nitrogenous matter present in the central canal. Denham also finds that some cottons are more resistant to attack than others, an observation to which it will be necessary to return later.

Denham's view that the attack on cotton hairs, and presumably on all fibres possessing a lumen, proceeds from the inside outwards has been accepted by Armstead and Harland<sup>7</sup>, Bright, Morris, and Summers<sup>4</sup>, and quite recently by Burns<sup>21</sup>, primarily on the theoretical consideration already outlined, that the development of the fungi ought to be favoured by an ample supply of nitrogenous material, but perhaps also on the assumption that the cuticle present on cotton hairs and many fibres would resist the attack. However logical these deductions may appear, it should not be forgotten that many lower fungi develop luxuriantly on inorganic nitrogen supplies or even, as already pointed out, in the absence of chemically combined nitrogen. Nor has it been proved experimentally that the cuticle is particularly resistant to fungi. An entirely satisfactory explanation of the important question as to where the attack starts has still to be supplied. It is felt that much valuable information could be obtained in this respect by determining in a sample of mildewed cotton fabric the ratio between those damaged hairs which contain hyphae in their central canals and those which do not.

In cases of such fibres and filaments as artificial silks, which do not possess a central canal, it may be taken for granted that the attack of the fungi proceeds from the outside inwards.

Whether starting from the outside of the hair or fibre and progressing inwards, or vice versa, the action of the fungi results in the tendering of the material, that is, in a reduction of its tensile strength and finally in its complete disintegration into a fine powder. In Denham's publication the tendering is described as a progressive splitting and shredding of the lamina of cellulose. In the case of what he terms 'dry' disintegration, that is presumably the destruction caused by fungi, as distinct from the wet disintegration for which bacteria are responsible, the broken ends of the hairs or fibres have a typical fibrillar appearance.

The biochemical and physical changes suffered by cellulose fibres and fabrics which have become tendered have hardly as yet been studied. The most complete information is contained in Cross and Bevan's report to the Indian Government on the heart damage of jute. These authors<sup>22</sup> found that the water solubility of the damaged fibres had been increased ten-fold, that their solubility in a 1 per cent. sodium hydroxide solution had been doubled, that their cellulose content had decreased from 75 per cent. to 62.6 per cent., and that the aqueous extract obtained from them reduced Fehling's solution. They describe the changes as an hydrolytic degeneration. From the data supplied by them it is clear that the decomposition follows the course of the usual decomposition of cellulose described in broad outlines in Chapter VIII. But why the decomposition should have given rise to the peculiar physical appearance of the damaged fibres described by Denham<sup>20</sup> and to their behaviour towards swelling reagents such as Schweizer's solution, first observed by Carbone<sup>23</sup>, still remains to be explained.

The prevention of the growth of fungi on fibres and fabrics is a problem which has occupied the attention of investigators from the time when the nature of mildewing was first established. And yet, as Bright, Morris, and Summers<sup>4</sup> very rightly

point out, practically no improvements have so far been made on the first suggested remedies—the addition of zinc chloride, phenol, or salicylic acid to the sizes—remedies which have proved far from reliable. The most successful treatment to-day would appear to be the coating of the fibres or hairs with copper salts of higher fatty acids, a treatment however which cannot be universally adopted, owing to the discoloration of the material thereby produced. The only really efficient treatment known, the removal of the moisture content of the fibres or its reduction to below the minimum admitting of the growth of fungi, is in many cases difficult or impossible to apply.

In the pages which follow it will be necessary to touch again upon the question of the prevention of the growth of micro-organisms on fibres and fabrics from certain specific points of view. Here it should be emphasized that much could be done in this direction by following Bright, Morris, and Summers's recommendation of creating conditions during the manufacture of textiles which would render it difficult for fungi to develop, and of preventing the spread of an already existing infection by introducing improved methods of cleanliness in textile mills. The selection of materials for the preparation of sizes which do not favour the growth of fungi would be a further step in the same direction.

Conditions favourable for the development of fungi also suffice for the growth of other cellulose-decomposing micro-organisms, particularly of actinomycetes. Bacteria probably require a somewhat higher moisture content than either fungi or actinomycetes, and it will be recollected that Denham refers to the destruction wrought by bacteria in cotton hairs as a wet disintegration. The moisture requirement of bacteria must nevertheless be easily satisfied, since Fleming and Thaysen<sup>10</sup> found a rapid increase in the number of bacteria contained in a sample of Indian raw cotton with 10 per cent. of moisture, when it was incubated for three days at room temperature. That bacteria in certain circumstances can be responsible for the decay of fibres and fabrics without the assistance of fungi is certain. Thus Cross and Bevan<sup>22</sup> found

no signs of fungi in the sample of heart-damaged jute which they investigated, and Dorée<sup>24</sup>, who studied the decay of fabrics in sea-water, describes it as the action of bacteria. In other cases, such as the destruction of Manila hemp described by Dorée<sup>13</sup> or of grey cloth studied by Osborn<sup>1</sup>, bacteria would appear to be associated with fungi in the decay. The relative importance of bacteria and fungi cannot at present be definitely circumscribed, since the investigation of the activity of the former in this direction has only recently been seriously considered and still remains incompletely studied. There can be no doubt, however, that the importance of bacteria is very great, particularly in the case of such materials as raw cotton and linen and of fabrics subjected to soil contamination or to exposure under water. Some measure of support for this statement can be obtained from a survey of the conditions under which the destruction of fibres and fabrics has been shown to be due in part at least to the action of bacteria. Among them may be mentioned the black arm cotton boll rot, described by Goulding<sup>25</sup> as due to the activity of *Bact. malvacearum*. This rot is first noticeable as a small dark brown patch on the boll at a point near the peduncle. If it starts some time before ripening takes place, the boll does not open and the immature hairs decay. If it occurs later, only part of the boll is affected and a certain amount of cotton may then be collected. In this case bacterial activity prevents the formation of cellulose rather than destroys it. But this is not so in the serious types of damage to which cotton, jute, and other fibres are exposed under damp conditions, for instance when stored in hydraulically pressed bales, which are deliberately damped to facilitate pressing, or for which insufficiently dried material has been used. Technically the damaged fibres are known as 'felted' cotton or 'heart-damaged' jute. Todd<sup>26</sup> in his text-book on cotton gives the following description of the origin of 'felting': 'It occurs when moisture enters the bale either during ginning or baling. In subsequent storage of the bale or its transport from its country of origin, the small spots develop into great lumps of rotten and felted cotton weighing many pounds.'



Heart-damaged jute is very similar in appearance and owes its origin to the same cause. Finlow<sup>27</sup>, in his comparatively recent investigation, found that the extent of heart damage is directly proportional to the amount of moisture present, and that the amount of moisture required to start the damage is inversely proportional to the pressure applied in the making of the bale. Under high pressure the decay may proceed at a moisture content of 25 per cent., while in the looser packed bales as much as 50 per cent. may be required. During the progress of heart damage the temperature of the jute bale rises to 40 C., and may remain at this figure for weeks. From this point of view the microbiological change resembles that occurring in spontaneously heated hay. The microflora responsible for the decay has still to be investigated. It is probable, as Cross and Bevan suggested, that the more important changes in the cellulose are due, both in this and in the case of felted cotton, to the action of anaerobic cellulose-decomposing bacteria.

The so-called 'country-damaged' cotton, for the formation of which Denham showed bacteria to be partly responsible, results from the exposure of cotton to damp before or after ginning. In extreme cases the cotton thereby becomes extensively tendered. Usually, however, country-damaged cotton has the appearance of an ordinary raw cotton with yellow or brown spots.

Another type of bacterial damage of cotton was recently described by Trotman and Sutton<sup>28</sup>. In a study of the weathering of textile fibres these authors observed that cotton incubated with *Bac. mesentericus* showed a swelling of the cell walls and a loss of natural twist. When cotton yarns were inoculated with a culture of the same organisms there was a slight increase in the tensile strength of the yarn during the first three days, followed by a rather marked decrease during the following days, making the yarn weaker than the original yarn after seven days' exposure. The same behaviour, though less pronounced, was found also in the case of yarns inoculated with a culture of *Bac. subtilis*. Trotman and Sutton's observations are very surprising, and undoubtedly

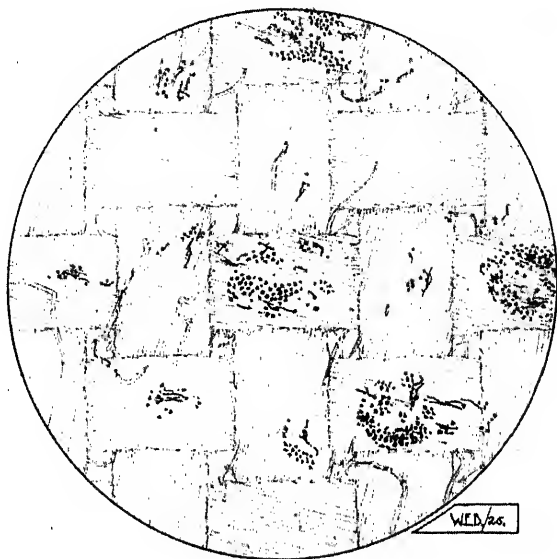


FIG. 10. Diagrammatic sketch of the mildewing of a cotton fabric as observed under the microscope with the low power.

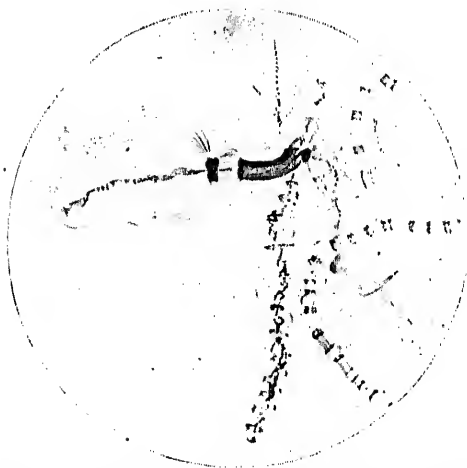


FIG. 11. Sample of normal cotton hairs, swelled by a mixture of carbon bisulphide and alkali. Magnification  $\times 90$ . (From N. Fleming and A. C. Thaysen, *Biochem. J.*, vol. 14, p. 25, 1920.)



need confirmation before they can be accepted, since neither *Bac. mesentericus* nor *Bac. subtilis* is able to decompose cellulose.

The bacterial infections to which flax is exposed are naturally very great and can hardly be avoided in view of the nature of most retting processes. The resulting decay has recently been investigated by von Gescher<sup>29</sup> and Ruschmann<sup>30</sup>. Ruschmann found that scutched flax which had been kept damp for fourteen days lost 25 per cent. of its tensile strength, and he expresses the view that the 12 per cent. of water which is regarded as a permissible moisture content for flax is too high. This undoubtedly is so, particularly as flax may be damaged not only through the destruction of its cellulose, but also by a resolution of the pectin which binds the individual flax fibres together, a decomposition which may be caused by a number of different bacteria, including such common types as *Bac. mesentericus* and *Bac. Megatherium*, which Ruschmann found extensively represented on scutched flax. Von Gescher in his investigations of flax damaged by micro-organisms paid particular attention to the cellulose-destroying bacteria, among which he describes a type which is either identical with, or closely related to, *Spirochaeta cytophaga*.

The destruction of tentage canvas through weathering was attributed by Ramsbottom<sup>18</sup> to both bacteria and fungi, and in the case of the rotting of fishing nets in the sea Dorée<sup>24</sup>, as already mentioned, found bacteria to be chiefly concerned.

The financial losses for which these various microbiological decomposition processes are responsible are difficult to estimate. That they are very large there can be no doubt. For jute, which is a comparatively unimportant textile fibre, Finlow<sup>27</sup> reports that in one year (1916) the losses through exposure to damp amounted to 3 per cent. of the total jute imports into Dundee.

The earlier attempts to study the microbiological decay of fibres and fabrics met with the serious difficulty that it was found impossible to determine the extent of the participation of cellulose-fermenting bacteria. There is still no known method by which these micro-organisms can be isolated and

enumerated, and deductions made as to the extent of their activity. Investigators seeking to establish the association of cellulose-decomposing bacteria with the decay have to rely upon indirect evidence, such as the arrest of the decay in the presence of antiseptics, or the abnormal increase of a secondary microflora present on the material, such an increase indicating the existence of conditions favourable to microbiological activity. It was with a view to overcoming this difficulty that Fleming and Thaysen<sup>6</sup> endeavoured to establish the participation of cellulose-decomposing bacteria in the destruction by direct microscopic examination of the decaying materials. As the examination of ordinary stained preparations did not lead to the desired results, they decided to make use of Cross and Bevan's viscose process<sup>31</sup>, which had previously been used by Balls<sup>32</sup> for the study of the detailed structure of the cotton hair. They expected in this way to magnify the changes in the structure of the decaying hairs to which the activity of the microflora had given rise. As applied for this purpose Fleming and Thaysen<sup>19</sup> give the following description of the method:

From 0.1 to 0.3 grm. of the fibres to be examined is placed in a small stoppered glass bottle with a wide mouth, and 10 c.cs. of 15 per cent. sodium hydroxide solution and the same quantity of carbon bisulphide added. The fibres are left in this mixture for 15 to 45 minutes, according to the degree of their decay, then removed to a microscope slide, covered with a cover-slip, and a drop of water allowed to diffuse under the latter.

Working with cotton hairs, a normal sample treated in this way had the appearance shown in Fig. 11.

Samples of cotton hairs which had previously been exposed to the action of micro-organisms behaved differently and gave the appearance illustrated in Figs. 12 and 13.

It will be noticed that the characteristic beading of normal cotton hairs, treated with the carbon bisulphide-alkali mixture, which is due to the resistance exercised by their cuticle against the expansion of the layers of cellulose enclosed by it, has entirely disappeared in those hairs which have been attacked by micro-organisms. Carbone<sup>23</sup>, as already mentioned, had observed this change some years earlier when swelling micro-



FIG. 12. Sample of cotton hairs, damaged by micro-organisms; after swelling by a mixture of carbon bisulphide and alkali. Magnification  $\times 100$ . (From N. Fleming and A. C. Thaysen, *Biochem. J.*, vol. 14, p. 25, 1920.)

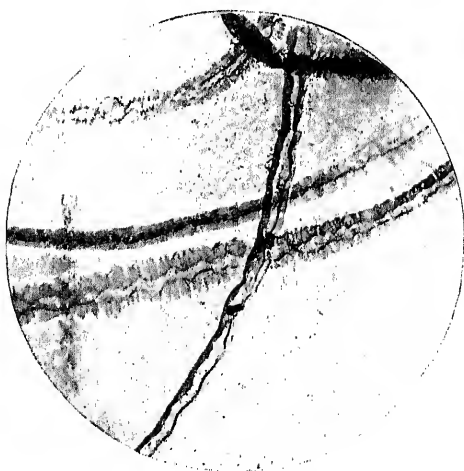


FIG. 13. Sample of cotton hairs, damaged by micro-organisms; after swelling by a mixture of carbon bisulphide and alkali. Magnification  $\times 100$ . (From N. Fleming and A. C. Thaysen, *Biochem. J.*, vol. 14, p. 25, 1920.)



biologically damaged cotton hairs with Schweizer's reagent. The impression is gained from a microscopic examination that the cuticle of the damaged hair has been completely destroyed or at least rendered incapable of resisting the swelling of the cellulose of the hair. In addition to the changes wrought in the cuticle the cellulose itself appears to have undergone changes. An inspection of Figs. 12 and 13 will show the frequent occurrence of incisions which are absent in normal hairs. It is on the basis of the presence of these rather than on the absence of beading that Fleming and Thaysen<sup>19</sup> devised a method for the quantitative determination of microbiological decay in cellulose hairs and fibres. Examination of fibres such as flax, jute, and wood-pulp showed that similar changes occurred in them after an attack by micro-organisms. Fleming and Thaysen do not mention, however, whether the changes they observed were typical of the action of micro-organisms, and were not produced by other agencies which tender fibres and fabrics, such as light and inorganic acids. This question, however, has been investigated recently by Searle<sup>33</sup>, who finds that, though difficult to discern for the untrained eye, a difference actually exists between the behaviour of flax fibres damaged by micro-organisms and material tendered by mechanical or chemical means, when swelled by the carbon bisulphide-alkali mixture.

The swelling test as described by Fleming and Thaysen is as follows:

As uniform a sample as possible is prepared from about 8 grms. of the material to be examined by carefully mixing it by hand. About 0.2 gm. of such a sample is mixed with 10 c.c.s. each of 15 per cent. sodium hydroxide solution and carbon bisulphide, and left to soak in the mixture with frequent shaking for a time sufficient to produce the required swelling of the hairs or fibres. When the degree of swelling has reached its optimum, a point which can be determined by examining small samples of the fibres under the microscope from time to time, three small samples of the swelled material, each the size of a large pea, are placed on three microscope slides. Each of these samples is again carefully mixed, for instance with the aid of two ordinary pins, and about twenty fibres, taken at random, spread out horizontally on each slide and covered with a cover-slip. A drop of water is allowed to diffuse beneath the cover-slips, and the slides are examined under the microscope.



Fig. 14 shows the appearance of a preparation made in this way. Ten counts are taken from each slide, the first running vertically along the right-hand edge of the cover-slip, the following parallel with, and to the left of the first, and so on. The ten counts should cover the whole of the preparation from the right-hand edge of the cover-slip to the left.

The average count from each of the three slides was found to vary little in well-mixed samples, an observation which, however, has recently been disputed by Burns<sup>21</sup>, who recommends the examination of a minimum of five slides. Burns also remarks that it is difficult to tease out from the 'pea' of swelled fibres a number of hairs or fibres which is sufficiently

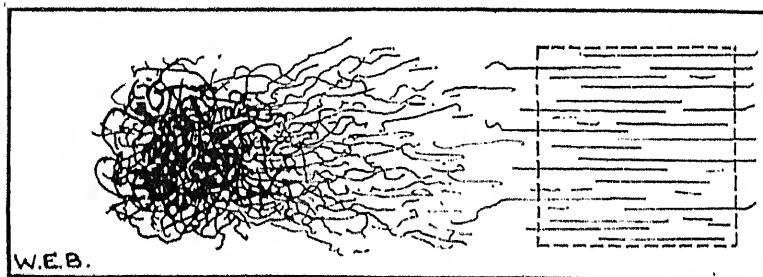


FIG. 14. Sketch showing the arrangement of cotton hairs on a microscope slide after swelling and spreading for the purpose of the quantitative determination of microbiological decay.

representative of the damage, when some of the fibres have been extensively decomposed and others little or not at all, and the material in consequence has had to remain in the swelling mixture for a prolonged time. In such cases he prefers to carry out the count on two parallel samples, one of which remains in the swelling mixture for the time sufficient to allow the badly damaged hairs or fibres to swell, while the other remains in the swelling mixture until the undamaged fibres have reached their optimum degree of expansion. The results obtained from the examination of five sets of slides of each of these samples Burns found to give a more reliable figure of the percentage deterioration than was obtainable by the procedure recommended by Fleming and Thaysen.

In order to obtain consistent results by this test, which Fleming and Thaysen termed the 'swelling test', it is of course necessary to lay down definite rules as to when a hair or a fibre is to be regarded as damaged and when as undamaged. Those who have studied the bacterial decay of fibres will have observed that the attack of the micro-organisms starts at isolated points and only gradually spreads throughout the length of the fibre or hair. The originators of the swelling test considered that a few isolated superficial wounds on an otherwise normal fibre would be unlikely to cause any measurable weakening in the strength of the fibres, and therefore decided to regard only those fibres, or isolated part of fibres, as damaged which showed signs of attack throughout their whole length. By choosing this standard they thought it probable that the results obtained by the swelling test would conform to the measurements obtainable by determining the tensile strength of the damaged hairs or fibres. Burns<sup>21</sup> very rightly remarks, however, that before this assumption can be admitted it is necessary to carry out determinations of the extent of damage in a number of fibre samples, both by the swelling test and by the measurement of the loss of tensile strength. Such comparative analyses are apparently now being undertaken by Burns and their results must be awaited with interest in view of the great value which attaches to the application of the swelling test to many problems connected with the microbiological decay of such fibres as cotton, flax, and wood cellulose, which are comparatively easily separated. Fibres such as jute Thaysen and Bunker<sup>34</sup> could not analyse quantitatively by the swelling test, owing to the difficulty of separating the fibre bundles.

With the swelling test, as first applied by Fleming and Thaysen, it was claimed that it was possible to detect a microbiological damage in artificial mixtures of normal and deteriorated cotton hairs, of as little as 2 per cent. of the total, and in a series of experiments in which the progress of the microbiological destruction of a sample of Indian raw cotton was followed, both by the swelling test and by the changes occurring in the alkali solubility of the attacked hairs,

a method frequently used for detecting tendering of textiles, the swelling test was found to be distinctly superior. While the swelling test showed a steady rise in the percentage of damaged hairs long before any change in the alkali solubility of the sample occurred, the latter method only gave definite signs of decay when this could already be detected by visual examination.

Soon after the publication of the first results obtained by the swelling test, it was criticized by Denham<sup>20</sup>, who maintained that hairs and fibres which had their cuticle damaged by mechanical means would behave as microbiologically damaged fibres when subjected to the swelling test, and that fibres and hairs in which the microbiological attack had started from the central canal would have the appearance of normal fibres when swelled by the carbon bisulphide alkali mixture. These objections were answered by Thaysen and Bunker<sup>24</sup>, who pointed out that the changes resulting from the microbiological decay could not have been restricted to a weakening, or perhaps even an elimination, of the cuticle, but would have involved also the cellulose of the material, since badly attacked fibres and fabrics showed a marked increase in alkali solubility. Further, they emphasized that the characteristic appearance of fibres and hairs after swelling is due as much to these changes in the cellulose as to changes in the cuticle. That a microbiological attack on a fibre or a cotton hair from the central canal should escape notice in the swelling test Thaysen and Bunker thought extremely unlikely, since such an attack would still involve a destruction of the cellulose. They admit, however, that they have seldom met with such cases, which according to them must be extremely rare. Wherever the fibres were found to be attacked by a fungus or a bacterium, either from within or from without, the swelling test was observed to record the damage. From this statement it is clear that the swelling test is applicable not only to fibres or hairs which have been bacterially damaged, but also to those which have been destroyed by fungi.

Despite the various criticisms levelled against the reliability of the swelling test, it is now generally admitted that it con-

stitutes a useful method for the quantitative estimation of microbiological destruction of many fibres and fabrics, and that the progress of such decay can be followed by means of it. It is interesting to consider therefore the observations made with the aid of the swelling test in Thaysen and Bunker's studies of the microbiological destruction of cellulose hairs and linen fibres. In a study of the rate of destruction of samples of American, Egyptian, Indian, and Indo-American

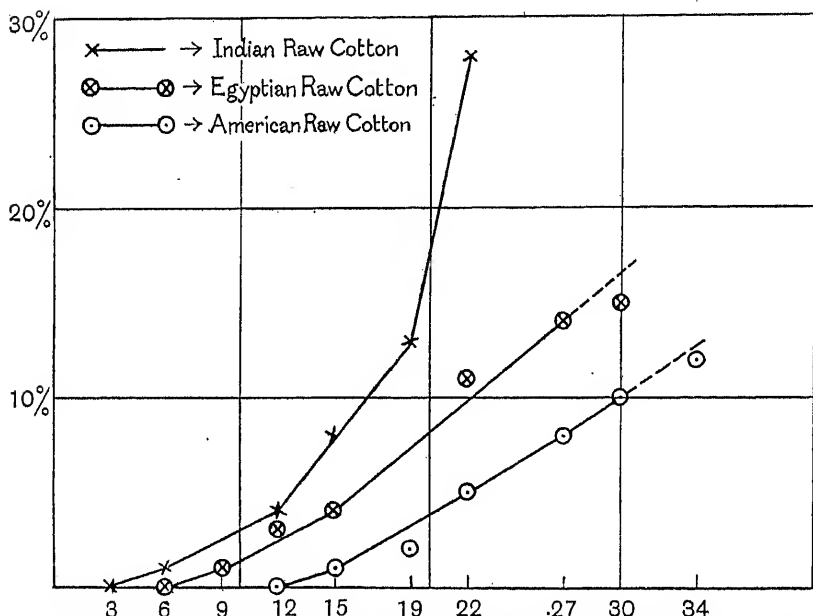


FIG. 15. Deterioration curves of Indian, Egyptian, and American cotton exposed to bacterial attack. The abscissae represent time in days. (From A. C. Thaysen and H. J. Bunker, *Biochem. J.*, vol. 18, p. 140, 1924.)

cotton it was found that the Indian samples decayed faster than any of the others, and that the American samples were the most resistant, followed closely by the Egyptian samples. The rate of decay of the Indo-American samples, that is of cotton grown in India from American seed, was found to be exceptional. It will be more fully referred to at a later stage. The rate of destruction of each of the first three types was found to be practically constant, so much so

in fact that on several occasions it was possible to identify raw cottons by means of their rate of destruction by micro-organisms. The accompanying diagram (Fig. 15) is reproduced from Thaysen and Bunker's publication and shows the rate of destruction of the three types of cotton expressed graphically.

Should these observations, that cottons of different origin show a difference in susceptibility to attack by micro-organisms, be confirmed on further investigation, it will be of very great theoretical and practical interest, since they offer a clue to the elucidation of the very important question of the prevention of the microbiological destruction of fibres and fabrics. Thaysen and Bunker realized this and made an attempt to find the reason for the differences observed. They directed their attention to ascertaining whether differences in the various samples of food available could be responsible for the differences in the rate of decay. They found, however, that an addition of food substances did not affect the ratio, except in the case of Indo-American cotton samples. Here, where the hairs normally decayed at the rate typical for American samples, the addition of a mixture of peptone and di-potassium hydrogen phosphate caused a considerable shortening of the time of exposure necessary to produce extensive damage in the samples.

The presence of inhibitory substances in American and Egyptian samples which could prevent the growth of micro-organisms and which might be thought responsible for the differences in rate of decay could not be confirmed experimentally. Nor was it possible to confirm the suggestion that the more resistant types of hairs possessed a cuticle with greater power of resistance to attack than that of Indian samples. The experiments in this direction were carried out on a sample of Egyptian combed Sakel sliver cotton, part of which was exposed to the action of micro-organisms without being subjected to any preliminary treatment, while other parts were first boiled in water to remove water-soluble compounds, or in a 3 per cent. sodium hydroxide solution under pressure to eliminate fatty or waxy compounds. The three sets of samples were all exposed under identically similar conditions,

and in the presence of additional food, consisting of a solution of peptone and di-potassium hydrogen phosphate. Contrary to expectations, the untreated sample was found to decay first, the sample treated with alkali under pressure showing less than half the damage of the untreated sample after thirty days' exposure. Burns<sup>21</sup> regards the result of this experiment as an indication that the attack of micro-organisms on the hairs proceeds from the inside of the central canal, and suggests that the treatment with alkali under pressure had removed the proteins or other food materials contained in the lumen and in consequence had rendered the development of micro-organisms in the central canal difficult. In forming this opinion Burns, however, has overlooked the fact that suitable food materials were purposely added to all the samples before their exposure, in order to counteract a possible destruction of the food normally contained in the hairs during their treatment with water or alkali. Thaysen and Bunker were therefore justified in concluding that the resistance of the slowly decaying types of cotton hairs has nothing to do with the presence of a particularly resistant cuticle.

The exceptional behaviour of typical Indo-American cotton samples towards microbiological decay was also studied in some detail by Thaysen and Bunker, who express the view that the decreased resistance to decay of these samples when additional food material is supplied must be ascribed to different climatic or soil conditions from those of their country of origin, and not to interbreeding with typical Indian cottons, since one of the Indo-American samples which they examined and which behaved exactly like all the others had, according to Burt<sup>35</sup>, been maintained in India as a pure strain of American cotton.

In their attempts to test the usefulness of the swelling test for the study of the rate of decay of other fibres, Thaysen and Bunker found that dew-retted flax showed a much slower rate of decay than tank-retted flax. Here again their observations, made on one series of experiments only, would appear to require confirmation. Taking into account, however, the differences in the process of retting of the two types of flax it

cannot be considered surprising that dew-retted flax should be more resistant than tank-retted flax. It may be recollected that Ruschmann<sup>14</sup> in his studies of dew-retting pointed out that *Cladosporium*, which he found to be chiefly responsible for the retting in this case, prevented the development of all other types of micro-organisms. It is quite conceivable, therefore, that the inhibitory substances produced by this fungus, and by means of which the growth of other micro-organisms was prevented during retting, may have survived and still have been present in the finished flax.

Before leaving the subject of the swelling test it should be mentioned that normal and damaged hairs or fibres can be swelled with a number of other reagents. The carbon bisulphide-alkali mixture, however, has the advantage over Schweizer's reagent and solutions of thiocyanates (Williams<sup>36</sup>) in that the swelling progresses more slowly and can therefore be studied more thoroughly under the microscope.

In discussing the mildewing of fibres and fabrics, it was pointed out that none of the methods recommended for the prevention of this destruction had proved really efficacious, except the reduction of the moisture content of the materials to a figure below 8 per cent. This is even more emphatically the case with the bacterial decay of fibres and fabrics, since the conditions under which fibres and fabrics undergo destruction by bacteria are frequently such that an application of antiseptics is rendered difficult. This is the case with fishing-nets, for instance, where the antiseptic will sooner or later be washed out through the repeated or prolonged wetting to which these fabrics are exposed.

Dry storage, of course, will protect any fibre or fabric against bacterial decay, a point which is emphasized by Burns<sup>21</sup>, who states that Egyptian cotton before ginning is more susceptible to decay than when damped after ginning, for instance, during pressing. He recommends the storage of cotton in well-ventilated warehouses and states that the best results are then obtained only when the cotton has been sun-dried before storage. Ventilation alone during storage, without preliminary drying, appears to favour the process of decay. This is cor-

roborated by observations already made in the case of the spontaneous combustion of hay.

The application of antiseptics for the prevention of bacterial decay has already been extensively tested, frequently with satisfactory results, as for instance in the case of canvas tents, tarpaulins, paper for roof covering, and fishing-nets.

Here the impregnation with copper salts would appear to be especially suitable, as in the Willesden treatment, where the copper salt is so intimately incorporated with the fibres that its removal by water is difficult. The bluish-green colour thereby imparted to the fibres, however, is frequently undesirable, and the general application of this otherwise effective treatment is therefore impossible. The same may be said of the impregnation with copper soaps, a treatment which has been recommended by Taylor and Wells<sup>37</sup> for the preservation of fishing-nets, the life of which is thereby materially prolonged. In the best of cases, however, such treatments must be considered as temporary expedients, which do not touch upon the fundamental principles underlying the susceptibility or resistance of organic materials to destruction by micro-organisms. Of far greater importance in this connexion are Doree's<sup>24</sup> observations that fabrics made from cellulose acetate are more resistant to microbiological destruction than ordinary cellulose fibres. Dorée suggests as an explanation that the reactivity of the hydroxyl groups of the cellulose molecule is lost by the replacement of their hydrogen by acetyl groups, and that in consequence the normal microbiological disintegration of the cellulose cannot take place.

Another, though still obscure, explanation is foreshadowed in experiments by Pringsheim and Aronowsky<sup>38</sup>. These authors found that inulin obtained by the saponification of inulin acetate, though possessing the chemical properties of normal inulin, was as difficult to decompose as inulin acetate by an enzyme prepared from *Penicillium glaucum*. In this case the recovery of the hydroxyl group after saponification of the inulin acetate had not restored the susceptibility of the carbohydrate to destruction by micro-organisms. An explanation of the greater resistance of the recovered inulin is not



offered by Pringsheim and Aronowsky, but it is fairly certain that it cannot be associated with changes in its chemical properties, since the recovered inulin was identical, chemically speaking, with ordinary inulin. Possibly the reason may have to be sought in physical changes such as those referred to by Herzog<sup>30</sup>, who found that of all the cellulose and cellulose derivatives examined cellulose acetate was the only one which possessed an amorphous structure.

The question of the microbiological destruction of artificial silk has recently been reviewed from this point of view by Thaysen and Bunker<sup>40</sup>.

Quite recent research by Pringsheim and Perewosky<sup>41</sup> has failed to confirm Pringsheim and Aronowsky's observations on the greater resistance to fungal decay of inulin prepared by the saponification of acetyl inulin. There is, therefore, at present no justification for the assumption that the changes wrought by this chemical manipulation of the polysaccharide can explain the greater resistance of cellulose acetate to microbiological destruction.

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## ADDENDUM

Since this chapter was written another method for the detection of microbiological decay in cotton hairs has been described by T. B. Bright (*J. Text. Inst.*, vol. 17, p. T 396, 1926).

## CHAPTER XII

### WOOD AND WOOD-PULP

THE account of the destruction of wood to be given in the following pages will be limited to a discussion of the action of micro-organisms on lumber, manufactured wood and wood-pulp, a subject which is now, in the literature of the last few decades, receiving a degree of attention commensurate with its economic importance.

Though most wood tissues are more or less heavily lignified, they are by no means safe against microbiological decay, and the cellulose, hemicelluloses, pectin, the carbohydrates dissolved or deposited in their parenchymatous tissues, and even the lignin can be decomposed by micro-organisms. The destruction of these substances is greatly facilitated by the presence in the wood of other substances which are essential for, or at least favourable to, the development of micro-organisms. Among such substances may be mentioned water, which occurs in undried woods to the extent of 20 to 60 per cent. (von Tubeuf<sup>1</sup>). Of these amounts even the minimum suffices to admit of the growth of many fungi. Nitrogen is present in the form of protoplasm, while the ash of wood contains all the salts necessary for the stimulation of growth.

The question of the nature of the organisms responsible for the rotting of wood has long been decided in favour of the higher fungi. A consensus of existing literature undoubtedly supports the view that these fungi are chiefly responsible for the decay, whereas the lower fungi and bacteria appear to play a more insignificant role. Only isolated references, such as those of van Iterson, jr.<sup>2</sup>, ascribe the decay of wood to the action of cellulose-decomposing bacteria, and in such cases abnormal conditions usually prevail. This was so in the case mentioned by van Iterson, where the decaying timber had

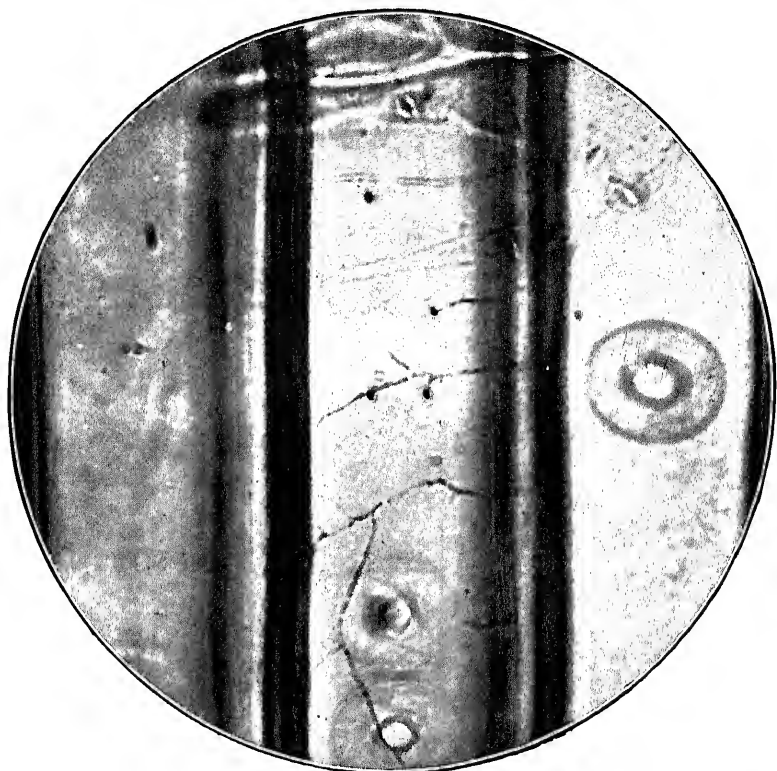


FIG. 16. Hyphae of *Polyporus schweinitzii* penetrating the cell wall of *Picea sitchensis*. Preparation stained with Bismarck brown and methyl violet. Magnification  $\times 1,300$ . (From Hubert, 'Diagnosis of Decay in Wood', *Journ. Agric. Research*, vol. 29, p. 523, 1924.)



been partly sunk in water and was found to rot on the border line between water and air, that is under water-logged conditions which most investigators have found unsuitable for the development of fungi. While touching upon the participation of bacteria in the decay of wood it should be mentioned that Schmitz<sup>3</sup> found that the presence of saprophytic bacteria in rotting wood very materially increased the rate of decay by fungi. The degree of this influence was found to vary with the type of bacterium present, with the species of fungus responsible for the decay, and with the type of wood attacked.

With practically no information available on the participation of bacteria the account to be given in the following pages of the decay of wood as manifested by the destruction of its cellulose, hemicelluloses, pectin, and gums must be a description of the action of fungi. The nature of these fungi and their position within the botanical system was discussed in Chapters V and VI.

In order to gain access to many of the substances contained in the wood, which may serve as food materials, the fungi must penetrate the wood tissues. They do this by means of the hyphae of their mycelium, which enter the wood, either through natural openings or through wounds, and gradually spread out between and through the cells. The intercellular development of the mycelium necessitates the resolution of the middle lamellae of the wood tissues, through the secretion of a pectosinase. The perforation of the cell walls, as Miyoshi<sup>4</sup> suggests, is probably due to a combined mechanical pressure exercised by the hyphae on the cell wall, and to the secretion of enzymes resolving lignocellulose. The holes produced in the cell walls by the perforating hyphae are an unmistakable sign of decay of the wood. Their significance in this respect will be discussed later. In Fig. 16, taken from Hubert's<sup>5</sup> paper on the diagnosis of the decay of wood, such perforations and the hyphae producing them are illustrated.

It is obvious that the thinner the cell walls of the attacked tissues the easier becomes their penetration by the hyphae. The sapwood, therefore, and particularly that part of it which is produced during spring, is generally more readily attacked

than the thick-walled heartwood, which as a rule is heavily encrusted with lignin and frequently filled with substances such as resins, tannin, colouring matter, and inorganic salts. For the same reason the medullary rays are usually the first tissues to be attacked and perforated, particularly by such fungi as *Ceratostomella pilifera*, which are unable to perforate lignified cell walls. Zeller's<sup>6</sup> investigation showed that the resistance of woods to destruction by fungi was proportional to the density of the wood, thus supporting the generally accepted view that heartwood is more resistant than sapwood.

Any resistant properties which the heartwood may possess do not protect it permanently from destruction by fungi. On being attacked it breaks down in the same way as the sapwood, leaving behind the same signs of decay which characterize the rotting of the latter. These signs are often characteristic of a given type of fungus and usually depend far more on the nature of the fungus than on the type of the wood. The identification of a given type of rot and of the responsible organism thereby becomes appreciably simplified. Thus some fungi, for instance *Trametes pini* and *Fomes igniarius*, decompose the lignin and leave behind patches of white or whitish cellulose, as shown in Fig. 17. Others, such as *Polyporus schweinitzii* and *Lenzites sepiaria*, decompose the cellulose and convert the wood into a brown brittle mass.

The so-called sap-stain fungi, to which *Ceratostomella pilifera* belongs, leave both the cellulose and the lignin almost undamaged, but invade the medullary rays and decompose the carbohydrates contained therein, at the same time imparting a discoloration to the attacked wood. In the specific case mentioned this discoloration is of a bluish grey colour. A few fungi developing on decaying wood render it phosphorescent. To these belong *Pleurotus Prometheus* and *Armillaria mellea*, investigated by Molisch<sup>7</sup>.

Following Hubert, the progress of the decay may be divided into two stages, the incipient stage and the typical stage. Of the two the former is in some ways the most important since it shows few if any visible signs of the presence of fungi, and is therefore difficult to diagnose. During the incipient stage

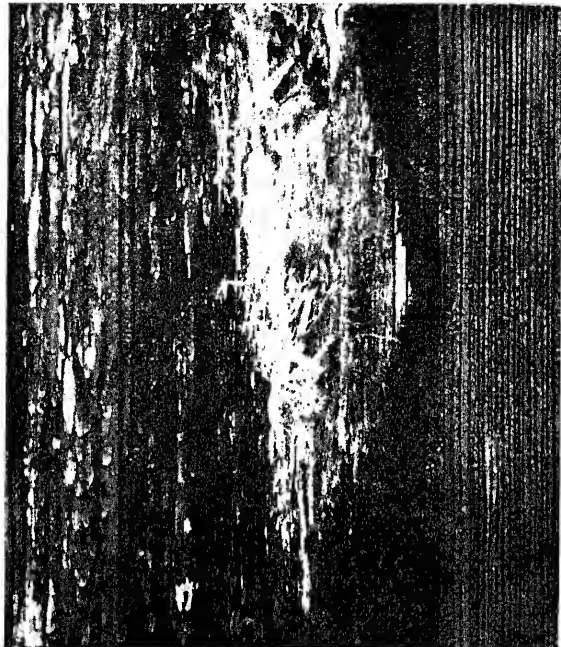


FIG. 17. Decomposition of larchwood by *Trametes pini*. In the centre, part of the wood has been converted into white cellulose. (Von Tubeuf in Lafar's *Handbuch der technischen Mykologie*.)

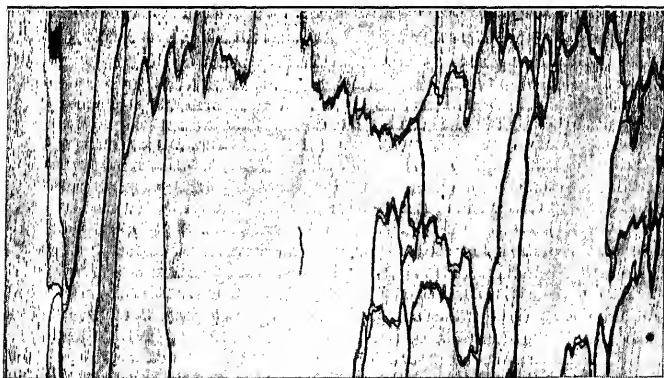


FIG. 18. Zone lines produced in a fallen trunk of *Acer rubrum* by *Xylaria polymorpha*. About  $\frac{4}{5}$  natural size. (From Hubert, 'The Diagnosis of Decay in Wood', *Journ. Agric. Research*, vol. 29, p. 523, 1924.)





the mycelium invades new tissue preparatory to a more extensive attack on the cells. The hyphae, which are hyaline, rather slender and seldom anastomosing, are often difficult to distinguish in unstained preparations (Hubert<sup>5</sup>). They ramify in all directions, and for preference seek the medullary rays and their readily decomposable content. Generally the wood retains its structural characters during this stage.

In cases of white rot, where the lignin becomes more or less completely decomposed, the beginning of decay may be signalized by the formation of zone lines, as shown in Fig. 18.

The dark coloration is produced round the area of fungus growth and may be seen in cross-sections of the dried attacked wood. The dark colour of the zone lines is described by Hubert as due to the oxidation of the decomposition products of the attack. This discoloration occurs on the exposure of the wood to the air during drying. During the incipient stage of decay tufts of mycelium may appear on the surface of the attacked wood (Mitchell<sup>8</sup>), as for instance in the mine timber rot caused by *Polyporus vaillantii*. Such mycelium may assist in the identification of the particular type of rot.

In the typical stage of decay the wood usually shows marked signs of loss of strength as well as changes of colour. This is due to the more or less complete resolution of its component parts and to the bleaching or pigmentation of the decaying tissues. The wood may become soft and spongy, or brittle and easily crushed, or it may be stringy as in the case of some white rots. In the rots caused by *Merulius lacrymans* and *Polyporus vaporarius*, where the comparatively thin-walled spring wood of each annual ring becomes severely damaged, the rotted wood forms a mass of brittle cubes. A case of this ring scale, or cubical rot, is illustrated in Fig. 19.

Another type of rot in its typical stage is shown in Fig. 20 and represents a brown pocket rot caused by *Polyporus amarus*. It demonstrates the restricted development of some timber rots.

In some cases, described by Mitchell in his paper already referred to, the attacked wood shows few gross changes even during the principal stage apart from the appearance of fruiting

bodies. Such cases, naturally, are particularly dangerous, since the wood, if used for strengthening purposes as in mine roof constructions, may suddenly and unexpectedly collapse.

The susceptibility to infection and the rate of decay of wood depend, apart from the nature of the attacking fungi, on a number of factors, including the type of wood attacked and the food substances present, and also the moisture and temperature conditions prevailing. Wood felled in spring and during early summer is particularly liable to attack by fungi if left on the forest floor without the removal of its bark to facilitate drying. The permeation during these seasons of all wood tissues by sap containing appreciable quantities of readily decomposable carbohydrates favours the development of a number of lower fungi which can hardly be regarded as wood-destroying forms (Haas<sup>9</sup>), but which discolour the wood beneath the bark with patches of black, yellow, pink, and green in a manner similar to that observed in samples of mildewed fabrics. The development both of these and of the true wood-destroying fungi depends, it need hardly be recalled, on the production of enzymes. Recent work, carried out primarily by American investigators, has established the great variety of the enzymes produced by one and the same type of fungus. Reference to this work was made in Chapter VI, where descriptions of the physiological properties of the saprophytic wood-destroying fungi were given. It was not recorded there, however, that the secretion of these enzymes is independent of the presence of the substances decomposable by them (Kylin<sup>10</sup>), though its extent may vary with the amount of substances available. This must be of the greatest importance for the rapid spread of the mycelium throughout its host.

The chemical changes produced in wood through the action of the various enzymes will be discussed later, when the conditions have been outlined under which an infection of wood may develop under ordinary conditions of storage and use.

That trees felled during spring are very liable to attack by fungi, particularly when left unbarked, has already been mentioned. But even carefully barked winter-felled trees

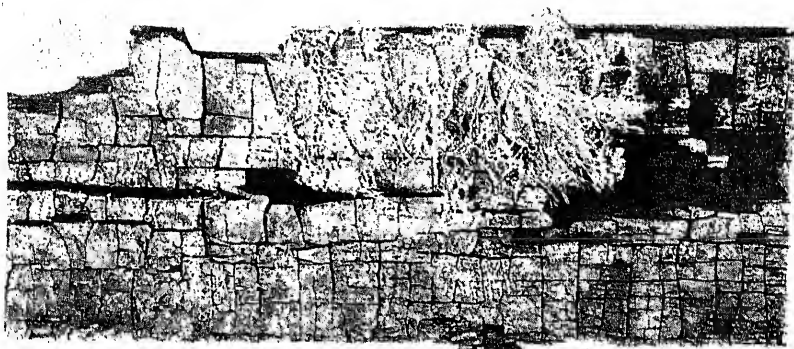


FIG. 19. Appearance of wood destroyed by *Merulius lacrymans*.  
(From von Tubenf, in Lafar's *Handbuch der technischen Mykologie*.)



FIG. 20. Brown pocket rot caused by *Polyporus amarus* in *Libocedrus decurrens*.  
(From Hubert, 'The Diagnosis of Decay in Wood',  
*Journ. Agric. Research*, vol. 29, p. 523, 1924.)



become attacked on storage, particularly when care is not taken to adjust the moisture conditions of the wood to within certain limits. These limits have been determined by a number of workers, among them by Hoxie<sup>11</sup> and by Snell<sup>12</sup>, who agree that a moisture content of 20 to 50 per cent. (Hoxie), or 25 to 42 per cent. (Snell), are those most favourable for the development of destructive fungi. For the typical timber rot caused by *Coniophora cerebella*, Scheible<sup>13</sup> finds an optimum moisture content of 50 to 60 per cent. The germination of the spores of wood-destroying fungi was studied by Zeller<sup>14</sup>, who found that the percentage germination of *Lenzites sepiaria* spores was low on wood with a moisture content below that of the point of fibre saturation, while it reached 100 per cent. in cases of wood fully saturated with water. On the basis of his observations Snell recommended the maintenance of the moisture content of stored wood either above 60 per cent., a water content which he considered sufficient to eliminate all danger of serious decay, or below 20 per cent. In view of this observation it is not surprising that storage under water should have been recommended as a safe method for the protection of timber against decay. Kress<sup>15</sup> is of the opinion, however, that storage in the open is preferable, provided that certain precautions are taken. He points out that though storage under water may be theoretically ideal, in practice it cannot very well be so, since many of the logs are bound to float on the surface of the water, thus becoming exposed to partial drying and consequent attack. For storage on land Kress recommends the erection of stacks separated from each other by passages at least three feet wide, each stack being so constructed that air is allowed free passage through the interior, presumably for the purpose of drying the stored wood. The stacks should not be placed on the ground, but on supports raising the bottom layers of timber sufficiently high off the ground to prevent their becoming damp. Lagerberg<sup>16</sup> agrees with Kress on the advantage of stacks and advises that the individual logs should not be longer than two metres, and that the bark should be as carefully removed as possible before stacking, so that the wood may become more readily

dried. But in spite of these precautions decay is likely to set in when infected, or perhaps even rotten, wood is allowed to remain in proximity to the stacked timber. This very frequently happens when infected timber is left scattered on the ground, a practice which might easily be avoided in the store yard and to a certain extent perhaps even in the forests, by the burning of all infected wood.

Far more difficult, of course, is the prevention of the spread of an already existing decay where timber is to be used in mines for the replacement of rotten props or roofs. Mine timber, therefore, frequently decays surprisingly quickly, often in a few months. Mitchell<sup>8</sup> estimates that about 50 per cent. of all timber used in mines in England and abroad is destroyed directly or indirectly as a result of fungal decay.

The financial losses incurred through the rotting of timber have not been determined, but must be very large indeed. An indication of their amount is given by Acree<sup>17</sup>, who, on the basis of replies to a *questionnaire*, places the annual losses of Canada through the fungal decay of timber at 5 to 10 million dollars. These figures are probably additional to the losses incurred through the destruction of building and other timber constructions by dry rot, *Merulius lacrymans*, and other types of rot such as those caused by *Polyporus vaporarius* and *Poria incrassata*, types which have often been mistaken for true dry rot. The two latter are probably unable to develop, however, under the restricted moisture conditions favourable to the typical dry rot.

On germination from its spore, for instance when present on a piece of timber touching a damp wall, the mycelium of *Merulius lacrymans* penetrates the cell walls of the attacked wood, seeking for preference the content of the medullary rays. The lignin of the cell tissues is not destroyed by the enzymes secreted, the action of its enzymes being largely restricted to the cellulose. The resolution and subsequent decomposition of the cellulose probably involves the formation of oxalic acid, since any calcium carbonate deposit present in the wood disappears as the decay progresses, and is found deposited as calcium oxalate on the rhizomorphs and the

ordinary mycelium. Macroscopically the attack of *Merulius lacrymans* first becomes noticeable as a brownish discoloration of the wood, the volume and weight of which decreases as the decay progresses. Hartig, quoted by von Tubeuf<sup>1</sup>, records a loss of volume of 25 per cent. in a piece of wood which had been acted upon for a year by *Merulius lacrymans*, while its loss in weight during the same period amounted to no less than 59 per cent. The shrinkage in all directions of the attacked wood is particularly noticeable on drying, when the timber shows the cubical appearance illustrated in Fig. 19.

The decomposed wood, though brittle and easily crumbled into a powder when dry, is hygroscopic, and the absorption of moisture causes the affected tissues to swell. Any excess of moisture absorbed is exuded from the living hyphae of the fungus and collects on them as tear-like droplets, an appearance which has given rise to the species name of this particular *Merulius* type. The formation of these droplets, which often consist of solutions of certain carbohydrates, also occurs in several other Polyporaceae, e.g. *Polyporus schweinitzii*.

The microscopic appearance and characteristics of the mycelium of *Merulius lacrymans* were described in detail in Chapter VI. Macroscopically the fungus may occur as white to greyish fan-shaped layers of mycelium adhering firmly to the wood. When food and moisture conditions are especially favourable the growth resembles a tuft of cotton-wool placed on the wood. From the layers of mycelium the fruiting bodies or sporophores of the fungus arise, but here, as in the case of many other wood-destroying forms, the mycelium may remain sterile and the identification of the fungus thus be rendered difficult. The reason why the mycelium remains sterile is perhaps the absence of light, which Long and Harsch<sup>18</sup> found, if not absolutely essential, at least highly favourable for the formation of sporophores in the case of many of the higher destructive fungi. The identification of the sterile mycelium of *Merulius lacrymans* is facilitated, Falek<sup>19</sup> suggests, by the fact that its mycelium ceases to grow at 27° C. The mature sporophore is amber-coloured or darker brown, covered with anastomosing wrinkles, over the surface



of which the basidiospores are formed. It measures from 10 to 20 cms. or more in diameter.

Particularly well developed in the case of *Merulius lacrymans* are the mycelial structures termed rhizomorphs, and it is due to the advanced development of this form of mycelium that the dry rot fungus is able to survive and to develop on what appears to be perfectly dry wood. The rhizomorphs consist of strands of closely interwoven hyphae containing between a network of narrow, thick-walled

hyphae several very wide cells, serving probably for the transport of water. A transverse section of a rhizomorph is shown in Fig. 21.

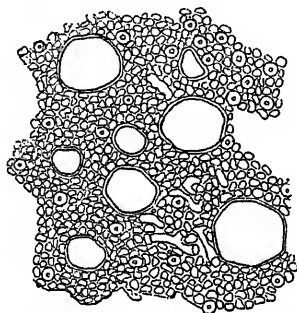


FIG. 21. *Merulius lacrymans*. Transverse section of a rhizomorph. Magnification  $\times 420$ .

(From Hartig, in von Tubeuf's article on wood-destroying fungi; Lafar's *Handbuch der technischen Mykologie*.)

The rhizomorphs spread for long distances away from the seat of the rot and may even penetrate brick walls and other obstacles in search of moisture. Rhizomorphs have also been observed in other

fungi which destroy wood, though they are not usually as elaborately differentiated as in the case of *Merulius lacrymans*.

*Polyporus vaporarius* and *Poria incrassata* are two other types of fungi which do serious damage by destroying timber in the forest as well as in storage yards and in buildings. Their moisture requirements are greater than those of *Merulius lacrymans*. Though the mycelium of **Polyporus vaporarius** greatly resembles that of the true dry rot fungus, its sporophores are sufficiently different to make the identification easy, being white and smaller, measuring from 2 cms. in length, and sometimes showing a yellowish tinge in the older parts. The rhizomorphs of *Polyporus vaporarius* are not nearly as elaborately differentiated as those of *Merulius*.

*lacrymans*. Wood attacked by *Polyporus vaporarius* becomes dark brown, almost charred in appearance.

The decay caused by *Poria incrassata* is similar, according to Humphrey<sup>20</sup>, to that produced by *Merulius lacrymans*, and is no doubt frequently confused with it, particularly when the mycelium present remains sterile. Infection starts in cool damp places, preferably on timber beneath floors which are in contact with the ground or close to it. The fungus develops most rapidly between the temperatures of 24 and 28° C.; below 24° C. growth is slower, but even at 12.8° C. it makes appreciable progress. It would appear to be particularly widespread in the United States, and attacks both coniferous and deciduous woods. Where the mycelium grows up through cracks in the affected wood it may form orange cushions. The fresh sporophores measure about 2 cms. in diameter and are at first orange-coloured to olive-grey. Later, when they harden and become leathery, they are sepia to brownish-black or liver-coloured. When young and succulent they are an easy prey to insects and bacteria, the former probably assisting in the spread of the fungus. Rhizomorphs may be present in this species, but, as in the case of *Polyporus vaporarius*, they are little differentiated. When young they are white, later becoming brownish-black. They adhere closely to the attacked wood.

Among other dangerous wood destroyers may be mentioned *Trametes pini*, *Coniophora cerebella*, *Lenzites sepiaria*, *Lenzites abietina*, *Stereum sanguinolentum*, *Paxillus panuoides*, and *Lentinus lepideus*. A fuller list of such fungi is given in Chapter VI.

The chemical changes occurring in wood as a result of its decay form an intensely interesting subject of study not only on account of their varied nature, but also because of their practical importance, and in view of the theoretical deductions which have been drawn from them by Fischer and Schrader<sup>21</sup> in their publications on the origin of coal.

The group of wood-stain fungi which limits its attack to the resolution of the cell content of the medullary rays and to a discolouration of the infected wood need not be considered

here, since these types do not seriously affect the hemicelluloses, cellulose or lignin.

The action of wood-destroying fungi on these substances has been the object of frequent investigations during the last decade and important deductions have been made from the results obtained. Usually, however, no account has been taken of the fact that the composition of the rotted wood largely depends on the particular type of fungus responsible for the decay. In reviewing the work already done it is essential to keep this fact in mind, since many of the results obtained are difficult to interpret on any other basis. Where it is a question of utilizing the results obtained for deductions of a theoretical nature future investigations should undoubtedly be limited to a study of wood decayed by pure cultures of fungi. Malenkovic<sup>22</sup> very ably emphasizes this in his studies of the action of *Coniophora cerebella*. The principle has been adhered to in some of the recent investigations, for instance, in those of Johnsen and Lee<sup>23</sup> and of Bray and Andrews<sup>24</sup>.

A fairly comprehensive account of the chemical changes occurring in wood as a result of its decay under natural conditions is given by Rose and Lisse<sup>25</sup>. These workers endeavoured to establish the changes involved by determining the effect of the decay upon the cellulose and the lignin by measuring the changes in solubility of the wood in hot and cold water, the increase in alkali solubility, the ether solubility, and the ash content after decay. For this purpose Rose and Lisse selected samples of Douglas fir, partly in a sound condition and partly in progressive stages of decay. The result of their analyses brings them to the conclusion that the changes occurring are progressive and very profound. Even where the wood had altered very little in structural appearance the chemical changes were easily measurable by analysis, an important point, no doubt, for the early diagnosis of the presence of decay in wood.

The cold-water soluble portion of the wood, doubtless consisting largely of water soluble carbohydrates, was found to decrease at an early stage in the decay, falling from 4.03 per cent. in sound Douglas fir to 1.76 per cent. in a nearly

decayed sample which had not been exposed to extraction by cold water during storage. In the completely rotted sample, which might have been thus exposed during storage, the cold-water soluble part still remained at 1.16 per cent.

The solubility, both in hot water and in dilute alkali, increased with the progress of the decay, the latter more than three times, from 10.61 to 38.1 per cent. in partly decayed wood. In completely rotted wood the alkali solubility reached a figure of 65.31 per cent. Rose and Lisse attribute this increase to a destruction of the cellulose content, which they found to decrease from 58.9 per cent. in the normal wood to 8.47 per cent. in the fully decayed sample. The pentosan content of the lignin fell from 7.16 per cent., through 6.79 per cent. in the partly decayed, to 2.96 per cent. in the completely rotted wood, while the methyl-pentosan and the methoxyl groups, calculated on the dry weight, increased respectively from 2.64 per cent., through 3.56 per cent., to 6.06 per cent. in the completely decayed samples, and from 3.94 per cent., through 5.16 per cent. to 7.8 per cent. This, of course, was not due to an actual production of these substances during decay, but to the rapid disappearance of other constituents of the wood. The figures, both for methyl pentosans and for the methoxyl groups, do not definitely prove that these substances had been left unattacked. On the contrary, the greater increase of methyl pentosan (more than 120 per cent.) as a result of the decay than of methoxyl groups (just under 100 per cent.) points to a slight destruction of the latter. Similar results were obtained by Mahood and Cable <sup>26</sup> in an analysis of a sample of ground wood-pulp attacked by *Paxillus panuoides*, and by Johnsen and Hovey <sup>27</sup>, who worked with wood attacked by *Fomes igniarius*. This fungus was stated by von Schrenk and Spaulding <sup>28</sup> to be responsible for the destruction of lignin in the attacked wood. Johnsen and Hovey <sup>27</sup>, however, find that this is not so, and that it is the cellulose which becomes decomposed, with a corresponding relative increase in the lignin content. That other constituents of wood may succumb to the enzymatic activity of fungi was shown by Acree <sup>17</sup>, who analysed spruce wood stored in stacks

for twelve months, and found that the wood had lost 75 per cent. of its methoxyl groups and 65 per cent. of its methyl pentosan, as well as 80 per cent. of its pentosan and 76 per cent. of its cellulose. At the same time the solubility in hot water had increased by 146 per cent. Bray and Andrews<sup>24</sup>, working with pure cultures, confirm the observations that part of the methoxyl groups are removed during decay and also point out that decayed wood reduces Fehling's solution more than does normal wood. This indicates an accumulation during decay of hexoses or pentoses or possibly of both. In the case examined by Bray and Andrews the methoxyl content fell to 2.8 per cent. as a result of the decay, a loss which, practically speaking, covered the loss of lignin—3 per cent.—observed by them. There are other cases on record, however, in which a far greater loss of lignin could be detected during decay and in which the cellulose content, calculated on the dry weight of the rotted wood, had materially increased. Thus Johnsen and Lee<sup>23</sup> found that a sample of wood destroyed by *Trametes pini* showed an increase in cellulose of 15 per cent., with a decrease in lignin of 30 per cent. A destruction of lignin, or of substances extracted from the lignin constituents of wood with hot alcohol, was observed by Gatin and Molliard<sup>29</sup> in the case of *Xylaria hypoxylon*. Though quantitative data are not available, it is to be expected that most, if not all, white rots are responsible for a similar decrease in the lignin content of the attacked wood, and probably for a corresponding increase in the cellulose content. It must be left to future investigations, however, to show whether such increase in the cellulose content corresponds to an increased yield of cellulose when white rotted wood is converted into chemical wood pulp. Johnsen and Lee<sup>23</sup> express the opinion that wood decayed by *Trametes pini* does give higher yields of sulphite pulp, but supply no experimental evidence in support of this statement.

In the vast majority of cases decayed wood, even in the incipient stage, is inferior to sound wood for all manufacturing purposes, including the production of wood-pulp and paper. This has repeatedly been shown during the last decade. The inferiority of the rotted wood is due not only to the loss of

substances incurred through decay, but equally, if not more so, to the chemical and physical changes of the remaining tissues, which have become incapable of withstanding the drastic processes to which wood has to be subjected for conversion into pulp. The inferiority of decayed wood is already noticeable in screening, when converted into chips preliminary to grinding. Here Kress, quoted by Johnsen and Lee<sup>23</sup>, found a loss of material of 5.62 per cent. in sound spruce, as compared with 13.22 per cent. to 15.6 per cent. in partly decayed, and 17.02 per cent. in badly rotted spruce. The grinding of the rotten chips gave rise to a much shorter fibre, in Kress, Humphrey, and Richards's<sup>30</sup> experiments, the fibre length averaging 0.25 mm. against 1.09 mm. in sound pulp. The paper made from the rotten pulp was dark coloured and of poor strength, the bursting strength being two and a half times less than that of paper made from sound wood. The losses of material on conversion into ground-wood paper were twice those incurred when using sound wood. The figures quoted relate, of course, only to the samples of decayed wood used in those particular cases, and they may be higher or lower, according to the degree of destruction shown by the wood. On the whole it is more reliable, therefore, to follow Hawkins<sup>31</sup>, and to determine the losses on samples of wood consisting of sound wood, but containing a definite percentage of completely decayed wood. In the case of a material of this description, containing 12 per cent. of rotten wood, Hawkins obtained 0.88 ton of ground pulp per cord as against 0.92 ton per cord of sound wood. On conversion into sulphite pulp the same sample yielded 0.483 ton per cord as against 0.542 ton per cord of sound wood. In spite of the greater losses incurred in the latter case, Hawkins, and many investigators with him, advocates the utilization of decayed wood for sulphite pulp preparation, rather than for ground wood-pulp, since the dark colour and the dirt, for which it is responsible, is less noticeable after chemical treatment than after mechanical preparation of the pulp.

In subjecting decayed wood to the sulphite treatment it should not be overlooked that a larger amount of chemicals is

required than in the case of sound wood. Sutermeister<sup>32</sup> reports that the increased cost in this respect varied from 0.6 to 1.6 per cent. for every 1 per cent. of rotted wood present. He, as well as Johnsen and Lee<sup>23</sup>, found that the bleached fibres prepared from rotted wood became more easily disintegrated—hydrated—than normal bleached fibres, and therefore suffered more under prolonged bleaching. He also records that the colour of the fibres prepared from decayed wood was much darker than that of fibres of sound wood, in spite of the use of a higher percentage of bleaching material in the former case.

The discoloration of the fibres of decayed wood is not due entirely to caramelization or charring of the cellulose during the chemical treatment. Howard<sup>33</sup> has shown that it may be the result of changes in the wood caused by the enzymes of the fungi. It is probable, therefore, that even wood which has suffered no more serious damage than a discoloration through the action of wood-stain fungi may be inferior as a raw material for paper making. McCubbin<sup>34</sup> suggests that a discoloration of the finished pulp may be traced to the presence of dark-coloured mycelial threads which have remained undecomposed in spite of the chemical treatment of the raw material.

While lower fungi such as *Alternaria*, *Stemphylium*, *Penicillium*, *Aspergillus*, and *Cladosporium* take little direct part in the destruction of timber and are present in rotten wood chiefly as secondary infections (Hubert<sup>5</sup>), living on the decomposition products of the wood destroyed by higher fungi, their importance becomes greatly increased when once the timber has been converted into pulp. Blair<sup>35</sup>, who studied the susceptibility of wood-pulp to destruction by lower fungi, found that all types of pulp, ground-wood as well as chemically treated pulp, were liable to attack. Incidentally, he points out that the mycelium 'in some cases' may be produced within the fibres, thus implying that it usually develops on their surface. The attack of the lower fungi originates, as in the case of the mildewing of fabrics, as isolated growths on the pulp, which gradually produce a dark grey, black, greenish, yellow, or even

deep pink discoloration. The pigments thus formed, according to Blair, are a source of serious damage, since they may spread throughout the pulp on its treatment preliminary to conversion into paper and thus cause a discoloration of the paper made from it.

As in the case of the destruction of the cellulose by higher fungi, the enzymes secreted by these lower forms frequently hydrate the cellulose of the pulp, converting it into cellobiose and glucose, and finally oxidizing it into carbon dioxide and water. The nature of these changes and their bearing on the physical properties of the damaged fibres have already been discussed in previous chapters.

A study of the microflora of paper was carried out by Sée<sup>36</sup>, who has also published a monograph<sup>37</sup> on the subject containing much important information. He has shown that only a comparatively small number of the numerous lower fungi, which from time to time have been stated to develop on paper, can be regarded as true paper inhabitants. Adopting a technique which excluded the infection of the examined samples by air-borne spores of micro-organisms, Sée obtained the following fungi from a number of different types of paper, including old manuscripts and books:

- Chaetomium Kunzeanum*, Zopf.
- „ *bostrychodes*, Zopf.
- „ *chartarum*, Berkeley.
- Myxotrichum chartarum*, Kunze.
- Eidamella spinosa*, Matruchot et Dassonville.
- Aspergillus sulphureus*, Desmazières.
- „ *brunneofuscus*, n. sp.
- Acrostalagmus cinnabarinus*, Corda.
- Spicaria elegans*, Corda, var. nov. *flava*.
- Cephalothecium roseum*, Corda, var.  $\beta$  *Matruchot*.
- Torula chartarum*, Link.
- Stachybotrys atra*, Corda.
- Dematium pullulans*, de Bary.
- Cladosporium herbarum*, Persoon.
- Stemphylium macrosporoideum*, Berkeley.
- „ *botryosum*, Wallroth.
- „ *piriforme*, Bonorden.
- „ *verruculosum*, Zimmermann.
- „ *graminis*, Corda.
- Alternaria polymorpha*, Planchon.



*Alternaria chartarum*, Preuss.  
    "    *varians*, Planchon.  
    "    *humicola*, Oudemans.  
*Stysanus stemonites*, Persoon.  
*Fusarium* species I.  
    "    species II.

In addition, one species of *Actinomyces* was isolated. The description given of this organism is, however, not sufficiently detailed for it to be classified.

Of the fungi mentioned, the *Stemphylium* and the *Cladosporium* species occurred most frequently. The *Chaetomium* species were also very common. The *Stachybotrys*, *Fusarium*, *Alternaria*, and *Stysanus* species, *Acrostalagmus cinnabarinus* and *Torula chartarum* were rather less frequent. *Spicaria elegans*, *Cephalothecium roseum*, and *Mycotrichum chartarum* were comparatively rare. *Dematium pullulans*, *Aspergillus brunneofuscus*, and *Aspergillus sulphureus* were only met with once.

A microscopic study of the paper samples on which these various micro-organisms were found showed that the organisms had been present in the paper during the process of its manufacture, entangled between the fibres, either in the form of mycelial threads or as spores. Their presence as mycelial threads also proved that conditions had been favourable for their growth prior to the drying of the paper, probably while still in the pulp stage. The time during which they could remain dormant in the finished paper must have been considerable and in some cases probably several years.

Their activity could be revived by placing the paper under suitable conditions of moisture and without any addition of food materials. Incubation of the infected papers at room temperature, and in suitable containers with distilled water, was sufficient to cause the pre-existing mycelium to spread and the spores to germinate. As a result, the paper became markedly mildewed within a few weeks.

Many of the fungi were found to produce characteristic soluble pigments which penetrated deeply into or through the paper, while in other cases the spores or the mycelium were characteristically coloured. The changes produced in the papers are shown in tabular form in Table II.

TABLE II

Type of fungus.	Discoloration produced.	Colour of pigment formed.
<i>Aspergillus brunneofuscus</i>	Purplish-black	Brown-red with violet shading
<i>Torula chartarum</i>	Soot black	Nil
<i>Alternaria chartarum</i>	Blackish-grey or blackish	Nil
<i>Stachybotrys atra</i>	Blackish-green or black	Greenish-grey or brownish
<i>Chaetomium bostrychodes</i>	Green-brown to deep olive	Yellowish
<i>Chaetomium chartarum</i>	Olive-brown with brownish black or blackish parts	Nil
<i>Chaetomium Kunzeanum</i>	Brownish-black	Yellowish-brown
<i>Cladosporium herbarum</i>	Blackish-grey, black or brownish-green	Nil
<i>Stemphylium macrosporoideum</i>	Very deep brown, at times almost black	{ Nil      Nil Yellowish or brownish, little marked Nil Nil
<i>Stemphylium botryosum</i>		
<i>Stemphylium piriforme</i>		
<i>Stemphylium verruculosum</i>		
<i>Stemphylium graminis</i>		
<i>Alternaria varians</i>		
<i>Alternaria humicola</i>		
<i>Stysanus stemonites</i>	Deep brown with black zones	Nil
<i>Alternaria polymorpha</i>		
<i>Myxotrichum chartarum</i>		
<i>Eidamella spinosa</i>	Deep brown	{ Yellow or brownish Nil
<i>Spicaria elegans</i> , var. <i>flava</i>	Light brown	Yellowish or very light brown
<i>Aspergillus sulphureus</i>	Yellow-brown	Yellow-brown or rust red
<i>Acrostalagmus cinnabarinus</i>	Yellow-red or ochre red	Yellowish to slightly orange
<i>Cephalothecium roseum</i> , var. <i>β Matruchot</i>	Tea-rose coloured to salmon pink	Yellowish and slightly pink
<i>Fusarium</i> No. II	Red, brown to deep brown	At first pink, then reddish-purple, rust red and finally brown

The remaining types did not show discoloration or pigmentation.

As a result of the attack, the paper became weakened and could in extreme cases be rubbed into a powder when dry. *Stachybotrys atra* appeared to be particularly active in this respect.

Having followed the destruction by micro-organisms to which wood is exposed from the felling of a tree to its conversion into paper, there remain to be discussed three questions connected with the decay to which sufficient attention has not yet been paid. One of these bears on the factors governing the rate of decay under natural conditions, the second on the methods available for the diagnosis of decay in wood, and the third on the identification of the fungi responsible for the decay.

Information gathered from the available literature shows that the rate of decay is intimately connected with the existence of suitable moisture and temperature conditions, but also indicates that it is dependent to some extent on the nature of the wood and on the responsible fungi, as well as on the reaction of the wood.

That a suitable temperature (not exceeding 35° C.) and moisture content (from 40 to 50 per cent.) are essential is obvious, and has already been emphasized. Under favourable conditions Mitchell<sup>8</sup> found mine timber completely destroyed in a few months. In tropical forests, where hot and moist conditions prevail, the decay of fallen trees no doubt proceeds at the same rapid rate, except when the fallen trunks become waterlogged. Then fungal activity becomes completely arrested. This is of the greatest importance in interpreting the part played by micro-organisms in the formation of peat and coal.

The effect of the nature of the wood on the rate of decay must be ascribed to the protective action of a heavy lignification (Zeller<sup>9</sup>), the rate of decay being inversely proportional to the density of the wood. Resin does not, according to this authority, possess antiseptic properties, but

it prevents the imbibition of water and thus retards the rate of decay.

The influence of the fungi on the rate of decay is governed by their power of secreting hydrolysing enzymes rather than by their rate of penetration of the attacked wood. For example, Lagerberg<sup>16</sup> found that *Stereum sanguinolentum*, which spreads very rapidly through wood, is comparatively slow in its destructive action. Secondary infections with saprophytic bacteria, which by themselves do not destroy wood, were found to increase the rate of decay materially in a case observed by Schmitz<sup>3</sup>.

The effect of the reaction of the wood on its rate of decay may be summarized by stating that though fungi prefer a slightly acid reaction, the presence of alkaline salts in small quantities stimulates the rate of decay. Such stimulation was observed by Schmitz<sup>38</sup> in the presence of sodium carbonate. When the reaction becomes too acid the rate of decay is adversely affected. Thus Meacham<sup>39</sup> observed a deflection in the growth-curve of wood-destroying fungi at a pH of 2.5, and a complete cessation of growth at a pH of 1.38. Since the decomposition of wood by fungi results in the production of organic acids, among them oxalic acid, it is probable that the stimulating effect of sodium carbonate observed by Schmitz may be ascribed to its neutralizing effect on these acids. A similar reason may be given for the favourable action on the rate of decay exercised by the presence of saprophytic bacteria.

Taking into account the influence of these various factors on the rate of decay it will be appreciated that no very reliable data can be supplied as to the time for which an infected piece of timber may be able to withstand the action of destructive fungi. Under one set of conditions it may be able to do so for years, whilst under others it may succumb in a few months. In a case examined by Bray and Andrews<sup>24</sup> fungi destroyed from 10.3 to 27.12 per cent. of the attacked wood in six months, 49.5 per cent. in twelve months, and 62.4 per cent. in three years. Somewhat more detailed data collected from laboratory experiments are supplied by Hubert<sup>5</sup>. They are recorded below in tabular form.

TABLE III  
Rate of Development of Decay in Wood.

Fungus.	Type of rot caused.	Host.	Date of inoculation of host.	Date of examination and result.
<i>Fomes igniarius</i>	White spongy rot	<i>Pinus strobus</i> + water	15.12.1922	15.5.1923, incipient rot and zone lines
<i>Fomes igniarius</i>	„	<i>Populus tremuloides</i> + water	15.12.1922	1.10.1923, incipient and typical rot, zone lines
<i>Fomes laricis</i>	Brown cubical rot	<i>Picea sitchensis</i> + water	17.3.1923	1.10.1923, incipient and typical rot
<i>Fomes laricis</i>	„	<i>Pinus strobus</i> + water	17.3.1923	1.10.1923, incipient and typical rot
<i>Fomes roseus</i>	„	Spruce + water	10.3.1921	February 1922, typical rot
<i>Ganoderma tsugae</i>	White pocket rot	<i>Picea sitchensis</i> + water	14.3.1923	24.10.1923, incipient and typical rot
<i>Hymenochaeta rubiginosa</i>	„	<i>Populus tremuloides</i>	14.4.1923	1.10.1923, incipient stage
<i>Lentinus lepideus</i>	Brown cubical rot	<i>Pinus strobus</i> + water	14.3.1923	1.10.1923, incipient and typical rot, badly decayed
<i>Lenzites sepiaria</i>	„	<i>Picea sitchensis</i> + water	15.12.1921	6.1.1922, typical rot
<i>Pholiota adiposa</i>	Brown mottled rot	<i>Tilia americana</i> + water	10.1.1922	3.3.1923, incipient and typical rot
<i>Pleurotus ostreatus</i>	White spongy rot	<i>Populus tremuloides</i> + water	15.12.1922	6.1.1923, typical rot and dimidiolate sporophores
<i>Polyporus adustus</i> (?)	„	<i>Populus tremuloides</i> , sapwood + water	17.3.1923	1.10.1923, incipient and typical rot, zone lines
<i>Polyporus adustus</i> (?)	„	<i>Populus tremuloides</i> , heartwood + water	17.3.1923	15.5.1923, typical rot and zone lines
<i>Polyporus amarus</i>	Brown pocket rot	<i>Picea sitchensis</i> + water	15.12.1922	15.5.1923, incipient discoloration, no typical rot

<i>Fungus.</i>	<i>Type of rot caused.</i>	<i>Host.</i>	<i>Date of inoculation of host.</i>	<i>Date of examination and result.</i>
<i>Polyporus anceps</i>	White pocket rot	<i>Picea canadensis</i> + water	15.12.1922	15.5.1923, typical rot and poroid growths shedding spores
<i>Polyporus balsameus</i>	Brown cubical rot	<i>Picea canadensis</i> + water	15.12.1922	15.5.1923, incipient and typical rot
<i>Polyporus ellisianus</i>	White pocket rot	<i>Picea sitchensis</i> + water	15.12.1922	1.10.1923, incipient and typical rot
<i>Polyporus pilosus</i>	"	"	14.3.1923	1.10.1923, incipient rot
<i>Polyporus schweinitzii</i>	Brown cubical rot	Spruce + water	15.12.1921	15.3.1923, incipient rot 1.10.1923, typical rot
<i>Polyporus</i> (= <i>Polystictus</i> ) <i>stipticus</i>	White pocket rot	<i>Picea canadensis</i> + water	3.4.1923	15.5.1923, typical rot and poroid growths
<i>Polyporus sulphureus</i>	Brown cubical rot	<i>Picea sitchensis</i> + water	15.12.1922	15.5.1923, incipient and typical rot
<i>Polyporus sulphureus</i>	"	Spruce + water	11.3.1922	18.11.1923, incipient and typical rot
<i>Polystictus versicolor</i>	White spongy rot	<i>Populus tremuloides</i> , sapwood + water	17.3.1923	17.5.1923, typical rot
<i>Stereum sulcatum</i>	White pocket rot	<i>Pinus strobus</i> + water	14.3.1923	8.1.1924, typical rot
<i>Trametes carnea</i>	Brown cubical rot	Spruce + water	15.12.1921	26.10.1922, typical rot and poroid growths
<i>Trametes carnea</i>	"	<i>Pinus strobus</i> + water	14.3.1923	1.10.1923, incipient and typical rot
<i>Trametes pini</i>	White pocket rot	Spruce + water	15.12.1921	26.10.1922, typical rot
<i>Trametes serialis</i>	Brown cubical rot	<i>Picea sitchensis</i> + water	15.12.1922	1.10.1923, incipient and typical rot and poroid growths

A second very important subject connected with the fungal destruction of wood is that of the methods available for the diagnosis of decay, particularly during its early or incipient stage, where structural changes in the wood are few and difficult to discover.

With the advent of aeroplane manufacture this subject has become particularly important, as infected wood must on no account be used in the construction of aircraft. A very interesting paper on the decay and discoloration of aeroplane wood has been published by Boyce<sup>40</sup>, who remarks that the first indication of decay usually consists of a discoloration of the infected wood, though all discolorations are not necessarily the result of decay. Thus marked discoloration, particularly of the sapwood of living trees, usually accompanies wounds caused by lightning, by larvae of insects living in the cambium during the growing season, and by sap-sucking birds. For practical purposes wood discoloured in this way is not reduced in strength.

The discoloration resulting from incipient fungal decay is found in both sapwood and in heartwood. Little is known, Boyce remarks, of the nature of the fungi causing decay in finished aeroplanes. Undoubtedly the chief forms are those commonly attacking manufactured wood products, e.g. *Merulius lacrymans*, the brown *Lenzites*, and the rose-coloured *Fomes*. Fungi decaying the heartwood of living trees are not commonly found in wood used for aeroplane structures. Where they do occur they are effective proof that the wood used was originally infected, and that sufficient care had not been exercised in eradicating the seat of infection. Decay in wooden aeroplane parts has become far more important in recent years, since large numbers of spare parts have often to be stored for a long time. Such parts should, in Boyce's opinion, be kept under conditions which will permit of good ventilation and will ensure that the moisture content of the wood does not exceed 11 per cent. Aeroplanes in use, quite apart from those employed in very damp tropical climates, are in parts exposed to decay, particularly near the engine and around the base of the wings, where an increased humidity and a somewhat higher temperature prevail.

The changes caused in wood through fungal attack are visible macroscopically when sufficiently advanced, but in the earlier stages are often only discernible under the microscope. Both the macroscopic and the microscopic changes may be made use of for the diagnosis of the decay.

As to the macroscopic changes, Hubert<sup>5</sup>, like Boyce, emphasizes the importance for the diagnosis of decay of the appearance of discoloration in suspected samples of wood. These discolorations are often striking, as for instance in the white rot caused by *Trametes pini*, where the heartwood becomes discoloured a dark red, brown, or even purplish tint during the incipient stage. In white rots, so termed because the attacking fungi destroy the lignin of the wood and leave the cellulose apparently undamaged, the discoloration often appears as zone rings delineating the extent of the activity of the invading mycelium. In brown rots, in which the cellulose of the wood becomes more or less completely decomposed while the lignin is little affected, zone lines are rare during the incipient stage (Hubert).

Zone lines may occur on the border line between the development of two separate fungi in cases where wood has suffered a mixed infection. In such cases they are broader than those occurring in white rots during the incipient stage. The appearance of zone lines is ascribed by Hubert to the oxidation and desiccation of the decomposition products of the destroyed wood. They are best seen in transverse dry sections of wood attacked by a white rot fungus (see Fig. 18).

Where the fungal attack causes no discoloration, changes in the texture of the wood may give information as to the presence of incipient decay. Thus sound wood, in Hubert's experience, pulls out with a more splintery appearance and shows greater elasticity than infected wood.

Indisputable evidence of decay is available where a microscopic examination of sections of wood reveals the presence of perforations of the cell walls, produced by the hyphae of wood-destroying fungi. A case of such perforation of the cell walls is shown in Fig. 16.

Since the structure of the perforations, or boreholes, is



frequently typical for the particular fungus producing them, their presence becomes a means not only of diagnosing decay, but also of assisting in the identification of the type of fungus responsible for the decay. Hubert records some interesting observations on this subject. Thus the boreholes may be small during the incipient stage and increase in diameter as the decay progresses. In other cases where that part of the hypha which occupies the borehole is constricted, the diameter of the hole changes little as it grows older. Here the perfora-

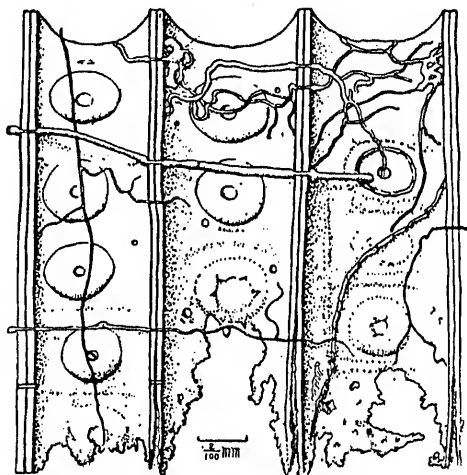


FIG. 22. Radial section through heartwood of *Pinus strobus* infected with *Trametes pini*. Magnification about  $\times 350$ . (From Hubert, 'The Diagnosis of Decay in Wood', *J. Agric. Res.*, vol. 29, p. 523, 1924.)

tions form cylinders of uniform diameter, and are placed perpendicularly to the surface of the cell walls. The white rot caused by *Trametes pini* shows this type of boreholes, which are illustrated in Fig. 22.

In other types of rot the boreholes have irregular outlines and often resemble an hour-glass; see Fig. 23.

The hour-glass shape seems to be a general feature in the case of boreholes which are twice to several times larger in diameter than the hyphae passing through them. Hubert

suggests that this peculiar shape is due to the greater resistance to decay of the middle lamellae of the cell walls, an explanation which does not appear satisfactory, since the middle lamella is usually recognized as the least resistant part of the cell wall. As the decay progresses the number of boreholes increases, and it is possible to judge the extent of the decay from their frequency.

Frequently the hyphae which have formed the boreholes are not visible during the incipient stage without special treatment owing to their hyaline nature. To facilitate their observation various staining methods have been suggested. Diemer and Gerry<sup>41</sup>, for instance, recommend the treatment of sections of the wood with a dilute solution of silver nitrate. This method, though excellent in the manner in which it differentiates the

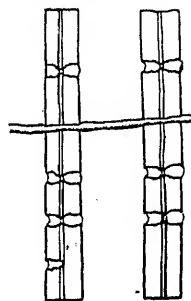


FIG. 28. Boreholes produced by *Fomes fomentarius* in *Betula papyrifera*. Magnification about  $\times 800$ . (From Hubert, 'The Diagnosis of Decay in Wood', *J. Agric. Res.*, vol. 29, p. 523, 1924.)

dark-stained, almost black, hyphae from the background of the more or less unstained wood, suffers from the disadvantage that it requires from twelve to twenty-five hours to carry out. Under routine conditions where a large number of wood samples have to be examined this is a distinct drawback. For such purposes Hubert<sup>42</sup> has devised a quicker staining method.

By this method the samples of wood, consisting of 1 c.c. pieces, are boiled in water for half an hour and then soaked until sufficiently soft in a mixture of equal parts of glycerine and 70 per cent. ethyl alcohol. Sections are cut from the cubes and treated as follows:

The sections are first flooded with a Bismarck brown solution (2 per cent. in 70 per cent. alcohol) for one to two minutes according to the type of wood and its density, the thickness of the sections, and the stage of decay. The excess stain is drained off, and the

sections washed with distilled water. They are then flooded for two to five minutes with a solution of methyl violet, made by mixing four parts of saturated aqueous solution of methyl violet with twelve parts of distilled water. In some cases it is necessary to use the methyl violet solution undiluted, and to stain for one to two minutes.

After staining, the sections are again washed in distilled water and examined, mounted in water, under the microscope. If the violet colour is found to be faint, the methyl violet staining process should be repeated. If the counter-stain (Bismarck brown) is faint, the whole procedure should be repeated. When the depth of stain is sufficient the sections are dried slowly on a warming plate after covering with a cover-glass to prevent curling. Curled sections may be fixed with egg albumen or gum arabic. The finished sections are mounted in balsam. On microscopic examination the hyphae will be found dyed a deep violet, while the cell walls of the wood are yellow to brown. Wood tissues showing exposed cellulose stain slightly with the methyl violet, giving a mixed brown and violet colour. The content of the medullary rays and the boarded pits of conifers usually stain a violet colour.

In addition to boreholes a microscopic examination of attacked wood will often show spiral cracks and corrosion marks in the cell walls, which Hubert considers valuable for diagnostic purposes, at least when hyphae are also present.

Though the chemical changes set up in an attacked sample of wood by the action of infecting fungi are limited to the area within the discoloured zones, the hyphae of the fungus often reach beyond them, sometimes for a considerable distance. By examination under the microscope and by cultural experiments with sections of attacked woods taken beyond the area of decay, Hubert established that the hyphae of *Polyporus schweinitzii* in *Picea sitchensis* may reach 63.5 cms. into the apparently sound wood. Other fungi, such as *Fomes igniarius* in *Populus tremuloides*, reach from 1.9 to 5.2 cms. beyond, while the hyphae of *Trametes pini* in *Pinus monticola* are confined within the zone of visible decay. Hence, when Boyce<sup>40</sup> suggests that the seat of infection may be effectively eradicated by the removal of all wood lying within 60 cms. in a longitudinal direction of the last visible signs of incipient decay, he does not allow a sufficient margin for such dangerous types of wood destroyers as *Polyporus schweinitzii*.

TABLE IV

*Diagnostic characters of various wood-destroying fungi.*

<i>Fungus.</i>	<i>White rot.</i>	<i>Brown rot.</i>	<i>Zone lines.</i>	<i>Large boreholes.</i>	<i>Small boreholes.</i>	<i>Spiral cracks.</i>	<i>Old hyphae not constricted (a).</i>	<i>Old hyphae constricted (a).</i>	<i>Old hyphae with little or no constriction.</i>	<i>Clamp connections noted.</i>	<i>Medullation hyphae.</i>	<i>Hyphae beyond incipient colour.</i>
<i>Armillaria mellea</i>	+	-	+	-	+	-	-	+	+	Nil	-	-
<i>Echinodontium tinctorum</i>	-	+	+	+	-	-	+	-	-	+	-	+
<i>Fomes annosus</i>	+	-	-	-	+	-	-	+	-	Nil	-	-
<i>Fomes applanatus</i>	+	-	+	-	+	-	-	+	-	-	-	+
<i>Fomes connatus</i>	+	-	+	+	-	-	+	-	-	+b	-	-
<i>Fomes everhartii</i>	+	-	+	-	-	-	-	-	-	-	-	-
<i>Fomes fomentarius</i>	+	-	+	+	-	-	+	-	-	Nil	-	-
<i>Fomes fraxinophilus</i>	+	-	+	+	-	-	+	-	-	+	-	-
<i>Fomes fulvus</i>	-	+	+	+	-	+	+	-	-	-	-	-
<i>Fomes igniarius</i>	+	-	+	+	-	-	+	-	-	Nil	-	-
<i>Fomes laricis</i>	-	+	-	+	-	+	+	-	-	+	-	+
<i>Fomesnigrolimitatus</i>	+	-	+	-	+	-	-	-	+	Nil	-	-
<i>Fomes pinicola</i>	-	+	-	+	-	+	+	-	-	+	-	+
<i>Fomes roseus</i>	-	+	-	+	-	+	+	-	-	+	-	+
<i>Ganoderma curtisii</i>	+	-	+	-	-	-	-	-	-	+	-	-
<i>Ganoderma tsugae</i>	+	-	+	-	-	-	+	+c	+	+	-	-
<i>Hydnum septentrionale</i>	+	-	+	+	-	-	+	-	-	Nil	-	-
<i>Hymenochaeta rubiginosa</i>	+	-	+	+d	+e	-	+	-	+e	Nil	-	-
<i>Lentinus lepideus</i>	-	+	-	+	-	+	+	-	-	+	+	+
<i>Lenzites betulina</i>	-	+	-	+	-	+	+	-	-	-	-	-

TABLE IV (continued)

Fungus.	White rot.	Brown rot.	Zone lines.	Large boreholes.	Small boreholes.	Spiral cracks.	Old hyphae not constricted (a).	Old hyphae constricted (a).	Old hyphae with little or no constriction.	Clamp connections noted.	Medallion hyphae.	Hyphae beyond incipient colour.
<i>Lenzites sepiaria</i>	-	+	-	+	-	+	+	-	-	+	+	-
<i>Merulius lacrymans</i>	-	+	-	+	-	+	+	-	-	+	-	-
<i>Pholiota adiposa</i>	-	+	-	-	+	-	-	+	-	Nil	-	-
<i>Pleurotus ostreatus</i>	+	-	-	+b	+e	-	+f	+e	-	Nil	-	-
<i>Pleurotus ulnarius</i>	-	+	-	+	-	+	+	-	-	Nil	-	-
<i>Polyporus adustus</i> (in <i>Populus</i> spec.)	+	-	-	+	-	-	+	-	-	+	-	-
<i>Polyporus amarus</i>	-	+	-	-	+	+	-	+	-	+	-	-
<i>Polyporus anceps</i>	+	-	-	-	+	-	-	+	-	+	-	-
<i>Polyporus balsameus</i>	-	+	-	+g	+	+	+g	+	-	+	-	+
<i>Polyporus berkeleyi</i>	-	+	-	+	-	-	+	-	-	+b	-	-
<i>Polyporus betulinus</i>	-	+	-	+	-	+	+	-	-	Nil	-	-
<i>Polyporus borealis</i>	+	-	-	-	+	-	-	+	-	Nil	-	-
<i>Polyporus circinatus</i>	+	-	-	+	-	-	+	+b	-	Nil	-	-
<i>Polyporus guttulatus</i>	-	+	-	+	-	+	+	-	-	Nil	-	-
<i>Polyporus resinosus</i>	+	-	+	+	-	-	+	-	-	Nil	-	-
<i>Polyporus robiniophilus</i>	+	-	+	+	-	-	+	-	-	Nil	-	+
<i>Polyporus schweinitzii</i>	-	+	-	-	+	+	-	+	+	Nil	-	+
<i>Polyporus squamosus</i>	+	-	-	+	-	-	+	+h	-	+	-	-
<i>Polyporus sulphureus</i>	-	+	-	+	-	+	+	-	-	+	-	-

TABLE IV (continued)

Fungus.	White rot.	Brown rot.	Zone lines.	Large boreholes.	Small boreholes.	Spiral cracks.	Old hyphae not constricted (a).	Old hyphae constricted (a).	Old hyphae with little or no constriction.	Clamp connexions noted.	Medallion hyphae.	Hyphae beyond anisopleni colour.
<i>Polystictus abietinus</i>	+	-	+	+	-	-	+	-	-	+	-	-
<i>Polystictus hirsutus</i>	+	-	-	+	-	-	+	-	-	Nil	-	-
<i>Polystictus pergamenus</i>	+	-	+	+	-	-	+	-	-	+	-	-
<i>Polystictus versicolor</i>	+	-	+	+	-	-	+	-	-	Nil	-	-
<i>Poria incrassata</i>	-	+	+	+	-	+	+	-	-	Nil	-	-
<i>Poria laevigata</i>	+	-	-	+	-	-	+	-	-	Nil	-	-
<i>Poria subacida</i>	+	-	-	-	+	-	-	+	-	+	-	-
<i>Stereum sulcatum</i>	+	-	+	-	+	-	-	+	-	Nil	-	-
<i>Trametes carneu</i>	-	+	-	+	-	+	+	-	-	+	-	+
<i>Trametes isabellina</i>	+	-	+	+	-	-	+	-	-	Nil	-	-
<i>Trametes malicola</i>	-	+	-	+	-	+	+	-	-	Nil	-	-
<i>Trametes pini</i>	+	-	+	-	+	-	-	+	-	+	-	-
<i>Trametes protracta</i>	-	+	-	+	-	+	+	-	-	-	-	-
<i>Trametes serialis</i>	-	+	-	+	-	-	+	-	-	+	+(?)	+
<i>Xylaria polymorpha</i>	+	-	+	+f	+e	-	+f	+e	-	Nil	-	-

(a) Passage of hyphae through cell walls of host.

(b) Rare.

(c) In summer wood.

(d) In vessels.

(e) In fibres.

(f) Pitted tubes.

(g) Late typical stage.

(h) Only when passing through heavily lignified walls (Buller).

A summary of some of the macroscopic and microscopic changes in wood caused by various fungi is given by Hubert and reproduced in Table IV on pp. 301-3.

A further means of diagnosing the presence of fungi in wood, both during the early and the later stages of decay, is now available in the various cultural methods which have reached a marked degree of perfection during the last few years. In carrying out these methods it should be remembered that wood in advanced stages of decay frequently contains a secondary microflora of lower fungi or bacteria often outnumbering that of the causative types of fungi.

A large number of different media, both of an inorganic and an organic nature, have been recommended from time to time for the cultivation of wood-destroying fungi.

Of those composed of inorganic salts, the best known is that recommended by Czapek (see Fritz<sup>43</sup>). It is used preferably with the addition of dextrose. In this form it has the following composition:

Magnesium sulphate . . . . .	0.5 gm.
Potassium di-hydrogen phosphate . . . . .	1.0 "
Potassium chloride . . . . .	0.5 "
Ferrous sulphate . . . . .	0.01 "
Sodium nitrate . . . . .	2.0 grms.
Dextrose . . . . .	30.0 "
Distilled water . . . . .	1 litre

The simplest and probably the most efficient all-round media are wort, strongly favoured by Hubert, and potato-dextrose decoction, equally strongly advocated by Fritz<sup>43</sup>.

The various media should for preference be used in the form of agar, which gives the necessary firmness for luxuriant and rapid development. Malenkovic<sup>22</sup>, in his study of *Coniophora cerebella*, strongly emphasizes the necessity for such support.

The method of preparation of wort and wort agar is that usually followed in microbiological technique, and need not be given here. Potato-dextrose agar may be prepared according to Fritz's formula:

*Potato-dextrose agar.* 100 grs. of sliced potatoes and 2 litres of distilled water are heated in an autoclave for thirty minutes at

15-lb. pressure and the liquid then strained. This potato decoction is used to replace the unabsorbed water drained from 25 grs. of shredded agar, which has been soaked overnight in 1 litre of distilled water. The potato decoction, with the soaked agar, is then heated in an autoclave for twenty minutes at 15-lb. pressure, and 25 grms. of dextrose added before the mixture is poured into flasks or tubes. The tubes or flasks containing the finished medium are sterilized for ten minutes at 15-lb. pressure.

Though most fungi prefer an acid reaction of the medium, their range of hydrogen ion concentration is sufficiently wide for an adjustment of most media to be unnecessary. When a secondary flora of bacteria is present, it is advisable to adjust the hydrogen ion concentration to a figure not exceeding pH 5.0 in order to check the development of this secondary flora.

The solid media are used either in test tubes as slopes or in Erlenmeyer flasks, with a shallow layer of the medium covering the bottom. To these tubes or flasks aseptically collected samples of the wood to be examined are added and slightly submerged in the solidifying medium. The temperature selected for incubation should not exceed 30° C. In general, 22° C. appears satisfactory (Fritz), though some forms such as *Fomes igniarius* may show an increased rate of growth and intensity of pigment production at 30° C. The presence of daylight during incubation, which Long and Harsch<sup>18</sup> found advantageous, at least for the formation of sporophores, is not necessary (Fritz) for the development of mycelium.

The isolation of the causative fungus in pure culture is most successfully achieved either from the spores of the sporophore or from wood in the early stages of decay, before the appearance of a secondary microflora. As a measure of protection against the appearance of infections, the first culture obtained from a sample of suspected wood can be purified by carefully transplanting selected pieces of mycelium to fresh media.

While the diagnosis of fungal decay in wood can to-day be established with a considerable degree of certainty, the subsequent identification of the causative fungus is still often a matter of great difficulty. Until quite recently such identification was usually possible only in the presence of sporophores,



since the identification was based on the nature of these bodies, on their presence in the proximity of the area of decay, and to some extent on the characteristics of the attacked wood. Fritz<sup>43</sup>, in her valuable paper on the cultural criteria available for the distinction of wood-destroying fungi, very rightly points out that the presence of a sporophore on the exterior of attacked wood is not necessarily a guarantee that this fruiting body has been produced by the mycelium causing the decay. It is conceivable also, as she points out, that different types of fungi may cause similar changes in an attacked wood.

Following Long and Harsch<sup>18</sup>, who were the first to probe the possibilities of constructing a system for the diagnosis of unknown rots on the basis of the diagnostic characteristics of their cultural properties, Fritz proposes to employ for this purpose, to a much greater extent than has hitherto been done, the peculiarities of the vegetative organs of wood-destroying fungi when grown in pure culture on artificial media. From the point of view of the microbiologist this proposal must be welcomed as being much more in keeping with general microbiological practice than the methods hitherto followed.

How successfully the diagnostic characters of pure cultures can be used for this purpose Fritz demonstrates in the case of those fungi which produce heart rot in *Abies balsamea*, a common rot met with in the forests of certain parts of Canada. Fritz's work will be quoted in some detail to illustrate the methods which must be adopted in extending the principle of wood-rot classification by cultural means.

The characters which may be of diagnostic value are both macroscopic and microscopic. The macroscopic features include the texture of the mycelium grown in pure culture, its colour, its rate of growth, and the manner in which it spreads over the surface of the agar. The microscopic characters comprise the types of hyphae, their colour, the method and frequency of their branching, their septation, the occurrence and type of clamp connexions, as well as the production of secondary spore forms. Other points such as the odour, typical for some forms, may also be considered.

In order to obtain results of value for comparative purposes,

standard methods were adopted by Fritz for the cultivation of the fungi. The medium selected as suitable from the point of view of easy preparation and general serviceability was potato-dextrose agar, prepared in the manner already described. The fungi were grown in test tubes measuring 2.3 by 15 cms., 15 c.cs. of the medium being contained in each. The solid medium was sloped to give the same area of surface in all cases. As temperature of incubation 22° C. was chosen. For convenience the cultures were incubated in the dark, since the characters of the fungi were found to be as well developed by this mode of incubation as when exposed to daylight for shorter or longer periods, as recommended by Long and Harsch<sup>18</sup>.

A summary of the diagnostic characters of those fungi studied by Fritz which may occur saprophytically is given in Chapter VI. The key constructed by Fritz for their classification is reproduced in full below.

*Key for the identification of the heart rots of Abies balsamea, based on the characters shown when grown on potato-dextrose agar.*

- I. Mycelium forming white or faintly yellow mats during one month's growth.
  - A. Mats radially furrowed : *Polystictus abietinus*.
  - B. Mats not furrowed.
    - (a) Chlamydospores present : *Polyporus borealis*.
    - (β) Chlamydospores absent.
      - (1) Fibre-like mycelial threads present.
        - (a) Fibres sparingly branched.
          - (o) Fibres uniform : *Poria subacida*.
          - (oo) Fibres with expansions : *Polystictus versicolor*.
        - (b) Fibres much branched : *Balsam rot type B* (2).
      - (2) Fibres absent.
        - (c) Clamp connexions always single : *Balsam rot type B* (3).
        - (d) Clamp connexions usually whorled : *Balsam rot type C* (1).

II. Mycelium forming faintly pink mats during one month's growth.

(a) Chlamydospores present: *Polyporus sulphureus*.

(β) Chlamydospores absent.

(c) Hyphae with clamp connexions very delicate: *Fomes pinicola*.

(.f) Hyphae with clamp connexions more heavily walled: *Fomes roseus*.

III. Mycelium forming mats variously and often deeply coloured, not as above.

(a) Chlamydospores present.

(g) Clamp connexions present: *Polyporus balsameus*.

(h) Clamp connexions absent: *Polyporus schweinitzii*.

(β) Chlamydospores absent.

(g) Clamp connexions present.

(x) Clamp connexions always single.

(y) Fibres coarse and darkly pigmented: *Fomes fomentarius*.

(yy) Fibres delicate, mostly hyaline: *Fomes applanatus*.

(xx) Clamp connexions usually whorled: *Balsam rot type A (2)*.

(h) Clamp connexions absent.

(s) Fibres uniform: *Fomes igniarius*.

(ss) Fibres with expansions: *Trametes pini*.

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PART FOUR

INDUSTRIAL APPLICATIONS



## CHAPTER XIII

# APPLICATION OF MICROBIOLOGICAL REACTIONS TO THE MANUFACTURE OF INDUSTRIAL COMPOUNDS FROM HEMICELLULOSES AND CELLULOSE

**The production of combustible gases.**—Though the marsh gas which escapes from the ground in some localities has been used as a combustible gas for domestic purposes for many years, and though numerous investigations have shown that the microbiological decay of vegetable debris frequently gives rise to the evolution of such gases, especially methane and hydrogen, few attempts have so far been made to utilize these reactions for the commercial production of gaseous fuels. Those made have not yet succeeded in establishing this industry, even in localities where vegetable debris is abundant and coal deficient. This, no doubt, is largely due to the existing imperfect knowledge of the microbiology of hemicelluloses and cellulose, and to the consequent difficulties encountered in attempting to control the breakdown of these substances on a technical scale. There is no *prima facie* reason to believe that such processes should necessarily be uneconomical. On the contrary, they appear to constitute a fruitful field for future investigations.

So far only two statements in the literature have dealt with this subject of the microbiological decomposition of cellulose from the point of view of its conversion into combustible gases. It is understood,\* however, that Viscount Elveden has designed a plant at Pyrford Court in which he has been able to produce combustible gases by the fermentation of straw, and that Amoore, working at the Rothamsted Experi-

\* Private information from Mr. E. H. Richards of the Rothamsted Experimental Station.



mental Station under the direction of Richards, has been able to produce methane on a technical scale from vegetable debris.

In 1918 Langwell and the Power Gas Corporation, Ltd.<sup>1</sup>, took out a patent which covered the manufacture of a combustible gas by the fermentation of cellulose as contained in straw and sulphite pulp. The experimental data on which this patent was based have since, it is understood, been subject to revision, but no published account is available showing how far the revised interpretation of the reactions holds out hope of their practical application.

The most recent experimental work on the subject dates from 1920 and was carried out by Fowler and Joshi<sup>2</sup>. The account of their experiments, however, overlooks a number of microbiological problems connected with the subject which must be solved before a serious attempt can be made to develop a process of this kind on economic lines. Some of these problems will be referred to below. In Fowler and Joshi's experiments an inoculant of sludge from a septic tank was used for the fermentation of the vegetable debris. This, added to a suspension of the debris in water, set up a fermentation and gas evolution which was particularly vigorous when the vegetable matter used consisted of banana and plantain skins, a material claimed to contain large quantities of hemicelluloses. Pure cellulose was found to be less satisfactory. A maximum production of gases was obtained when the fermentation was conducted at a surrounding temperature of 35° C. It was found advantageous occasionally to replace the water covering the fermenting material, and thus to reduce the concentration of the organic acids formed during fermentation to below 1.0 per cent. In such circumstances it was possible to secure a daily evolution of combustible gases equal to 80 per cent. of the volume occupied by the fermenting material. It is not stated for how long this rate of evolution could be maintained. The gases evolved contained from 80 to 85 per cent. of methane and their calorific value is claimed to have been 1.45 times that of ordinary coal gas.

For the purpose of comparison it is interesting to quote the figures obtained by Omelianski<sup>3</sup> in fermentation experiments

carried out with *Bac. methanigenes*, isolated by him from similar material. The figures refer to a series of laboratory experiments, in which resistant cellulose was used as the source of carbohydrates. To the filter paper contained in a 500 c.cs. flask, a food solution was added consisting of inorganic salts and water, with calcium carbonate to neutralize the organic acids produced during fermentation. The flask was inoculated with a small piece of decayed filter paper containing an almost pure culture of *Bac. methanigenes*. The incubation period was remarkably long, and not until after 29 days did the evolution of gas become visible. At this time the quantity of gas collected during 24 hours amounted to 2.4 c.cs. from the 500 c.cs. of fermenting liquid used. During the following four days the rate increased rapidly and reached a maximum of 26.2 c.cs. in 24 hours or 5.24 per cent. of the volume of the fermenting liquid. From this maximum the rate decreased fairly rapidly, and soon reached a minimum of 0.24 c.cs. per day, at which figure it stayed during the remaining three and a half months of the experiment. Altogether 552.2 c.cs. of mixed gases were given off from 2.0815 grammes of cellulose, consisting of 190.8 c.cs. (= 0.1372 gramme) of methane and 361.4 c.cs. (= 0.7146 gramme) of carbon dioxide. The volume of methane thus amounted to only 34.5 per cent. of the total, against 80 to 85 per cent. in Fowler and Joshi's experiments. The calorific value of the gas mixture obtained by Omelianski can only have been half that of ordinary coal gas.

The difference in yields in Fowler and Joshi's and in Omelianski's experiments are so striking that it must be assumed that the former were working with a type of cellulose decomposer other than *Bac. methanigenes*. Unfortunately they do not give any information as to the nature of the type used by them. Their results demonstrate the importance of selecting a type which not only gives a high yield of combustible gases, but which will maintain a maximum rate of gas evolution over a considerable period, preferably until the bulk of the vegetable debris has been decomposed. In this direction further investigations are required. The utilization of thermo-tolerant or thermophilic types, which decompose cellulose in

as many days as the mesophilic forms require weeks, might be particularly suitable in this respect.

It is necessary also to decide whether or not the organism selected should be used in pure culture and whether the presence of a secondary microflora would have a beneficial or a deleterious effect on the rate of gas evolution. In this connexion it should be mentioned that Khouvine<sup>4</sup> found that the rate of cellulose decomposition by *Bac. cellulosa* *dissolvens* was increased about five times in the presence of certain other bacteria. It would be desirable also to select a type which would not show that marked preference for hemicelluloses which Fowler and Joshi found their inoculant to have, but which would as readily decompose both cellulose and lignocellulose, the two substances which are most abundantly represented in vegetable tissues. This latter desideratum may not, of course, be feasible.

Another important problem to which attention should be directed is that of the conversion of the organic acids of the cellulose fermentation into further supplies of combustible gases. That this is possible is clear from the work already done on the fermentability of these acids. Thus Hoppe-Seyler<sup>5</sup> showed that calcium acetate solutions inoculated with sewage sludge yielded a gas consisting of one part of carbon dioxide and two parts of methane. From both potassium acetate and sodium butyrate Mazé<sup>6</sup> obtained a mixture of methane and carbon dioxide when inoculating solutions of these salts with a culture of a bacterium, termed *Pseudosarcina*, which he had isolated from leaves. Omelianski<sup>7</sup> recorded the evolution of a mixture of carbon dioxide and methane from calcium acetate when the solutions of this salt were inoculated with material from the bottom layer of a manure heap or from soil. The methane under favourable conditions amounted to as much as 98 per cent. of the total gas evolved. It would be necessary also to determine to what extent the rate of gas evolution is influenced by the addition of nitrogen and other food materials, and, if favourably affected, to establish how such food could be supplied economically.

Many other problems would undoubtedly require investiga-

tion before the microbiological conversion of waste vegetable matter into combustible gases could be claimed to have been scientifically explored. But the advantages accruing to many localities from such a process would be so valuable that a serious effort to solve them appears well worth while, particularly as any methane thus prepared might have other important uses, e. g. as a raw material for the manufacture of formaldehyde and methyl alcohol.

Perhaps the investigations, referred to by Nathan<sup>8</sup> as having been commenced at Nobel's research department at Ardeer for the production of methane by fermentation, may be a step in this direction.

**The production of power alcohol.**—A far more important problem, but one which is also infinitely more difficult to solve, is that of the conversion of the hemicelluloses and the cellulose present in vegetable waste materials into ethyl alcohol, entirely by microbiological reactions, without the application of any process of chemical hydrolysis.

During recent years this problem has been the subject of much comment owing to the desirability of economizing the existing, far from inexhaustible, supplies of petrol by the use of ethyl alcohol as a fuel for internal combustion engines. Such alcohol might, of course, be obtained from grain and other starch- or sugar-containing materials, but public opinion has lately been against the use of potential food materials for this purpose.

Theoretically, waste vegetable matter might be converted into ethyl alcohol in two ways, either by subjecting it to a preliminary hydrolysis with mineral acids, and a subsequent fermentation of the resulting monoses, or by submitting it to the action of alcohol-producing micro-organisms without previous hydrolysis. The former procedure has already met with a certain measure of success. It will not be discussed here, since it is outside the scope of the present inquiry. The second method, which obviates the hydrolysis, would undoubtedly be more advantageous if the serious difficulties could be overcome which at present prevent its practical application. These difficulties are in large measure due

to the complicated composition of most waste vegetable matter, which militates against its complete conversion into ethyl alcohol by simple microbiological processes. Thus while one type of organism would be required to convert the cellulose proper, another would probably be needed for the conversion of the lignified cellulose, while the hemicelluloses might require a third type and other substances a fourth or perhaps even a fifth. Complications might arise also from the antiseptic properties of tannins and similar substances present in the waste material, and from a mutually antagonistic action of the various micro-organisms used. Pure culture work with one or two types might therefore be difficult to apply, a serious complication, which, with our present knowledge, would prevent the proper control of the fermentation process.

Until such time as these difficulties have been mastered it is necessary to limit the microbiological production of alcohol from waste vegetable matter to a conversion of the cellulose, the hemicelluloses, or the lignocellulose present. The first aim must be, therefore, to secure a raw material in which one of these substances preponderates. A certain amount of work has already been done from this point of view in the case of hemicelluloses and of pure cellulose.

The work on the production of ethyl alcohol from hemicelluloses was carried out by Mezzadrolì<sup>9</sup>, who found that fungi of the type of *Mucor Rouxianus* could be used to hydrolyse waste supplies of vegetable ivory, the endosperm of *Phytelephas macrocarpa*, into mannose, which in turn could be fermented by yeast into ethyl alcohol. Mezzadrolì points out, however, that the conversion of the hemicellulose is slow; too slow, probably, for a practical application of the process. Moreover, the available supplies of waste vegetable ivory are far too small for a technical process for power alcohol production to be based on this raw material. Far more promising would be the use of xylan for this purpose, a hemicellulose which is abundantly represented in many types of waste vegetation. So far, however, its direct conversion into ethyl alcohol has not been achieved. Its hydrolysis and subsequent decomposition by fungi has been studied by Schmidt, Peterson, and Fred<sup>10</sup>,

who allowed various species of *Aspergillus* and *Penicillium*, as well as *Rhizopus nigricans*, to act upon maize- and rye-straw pentosans. Schmidt, Peterson, and Fred's work was referred to in Chapter V. The destruction of the pentosans was slow, continuing for two to several months, and it never involved more than 53 per cent. of the pentosans present in the straw, being in most cases limited to about 30 per cent. It is not likely, therefore, that a compound fermentation process, based on the use of such fungi for the hydrolysis of the pentosans, and of pentose-fermenting, alcohol-producing bacteria for the conversion of the hydrolysed pentosans, will offer a practical solution of the problem of the production of power alcohol from pentosans.

That alcohols are formed in the fermentation of cellulose by pure cultures of bacteria was first reported by Omelianski<sup>11</sup>, who detected traces of these substances in the liquid in which cellulose had been decomposed by *Bac. fossilicularum*. Previously van Senus<sup>12</sup> had suggested that the hydrogen evolved during the anaerobic fermentation of cellulose was capable of reducing part of the acetic acid formed to ethyl alcohol.

A quantitative determination of the ethyl alcohol resulting from the fermentation of cellulose by *Bac. cellulosa dissolvens* was carried out by Khouvine<sup>4</sup>, who obtained a yield of 8.1 per cent. of the cellulose decomposed. As only 55 per cent. of the carbon contained in the cellulose subjected to fermentation could be recovered in the decomposition products, the yield of ethyl alcohol, calculated on the cellulose used, was actually much lower.

Greater yields are reported by Fred, Peterson, and Viljoen<sup>13</sup>, who subjected cellulose to destruction by a thermophilic bacillus. The fermentation was carried out at 62 to 66° C. and was marked by a rapid evolution of gas, which finally became so violent that the cellulose pulp used was carried to the surface of the fermenting liquid. After three to four days the fermentation began to slow up. It was completed in four to six days, 60 to 80 per cent. of the cellulose having by then been decomposed.

The nature of the gas evolved is not recorded by Fred and

his collaborators. The other fermentation products consisted chiefly of acetic acid and ethyl alcohol, 56 per cent. of the cellulose fermented being converted into acetic acid and 10 per cent. into ethyl alcohol. Judging by the experiments recorded, the yields of the two chief fermentation products varied considerably. The ethyl alcohol formed, calculated on the cellulose fermented, was sometimes as low as 5 per cent., and at other times as high as 25 per cent. The yield of acetic acid fluctuated between 19.6 and 56.8 per cent. This variation in yield points to an insufficient control over the reactions occurring during fermentation, or possibly to the presence of foreign bacteria in the fermenting liquid. In a culture of this organism which the writers obtained from the American authors, infection forms were undoubtedly present. The visible contamination of this culture may have been responsible for the very low yields of ethyl alcohol, amounting to mere traces, which were obtained with it.

Fred and his collaborators also report on the fermentation of corn cobs by their thermophilic cellulose decomposer, but the yields of alcohol were low with this raw material, amounting only to 2.2 to 2.9 per cent. on the corn cobs taken, while the acetic acid produced varied between 14.4 and 26.7 per cent. Though a comparison of the yields obtained from cellulose and from corn cobs may not be possible in view of the different nature of the two types of raw material, it is surprising to note that the yields of organic acids from corn cobs, assuming these to have been fermented to the same extent as the cellulose, should have been no less than half, while the yield of alcohol in the case of the corn cobs was less than 15 per cent. of that obtained from the cellulose.

There are evidently a good many problems to be investigated in connexion with the use of this and other similar thermophilic micro-organisms before it is possible to decide whether such types can eventually be used for the production of ethyl alcohol from cellulose. In principle the use of thermophilic rather than of mesophilic forms is undoubtedly sound, since the former are much more rapid in their action on the raw material than the latter.

From a technical standpoint it would be an advantage also to be able to conduct the fermentation of the raw material direct without the aid of other micro-organisms for the preliminary conversion of the polysaccharides into monoses.

The outline given above of the work so far done on the production of power alcohol by direct fermentation shows that under certain conditions at least one type of hemicellulose, as well as pure cellulose, may be converted into ethyl alcohol. How far lignified cellulose can be similarly converted has not yet been established. As it constitutes by far the largest proportion of most, if not of all, the readily accessible vegetable waste materials, it would be of particular importance to determine its behaviour towards alcohol-producing micro-organisms, both from the point of view of its direct conversion and of its hydrolysis by fungi and subsequent fermentation by yeast or bacteria. In considering the possibilities of the latter method it should not be overlooked that some wood-destroying fungi, such as *Polyporus schweinitzii*, *Polyporus sulphureus*, and *Fomes pinicola*, exude from their sporophores droplets of liquid containing melezitose and mycose, soluble carbohydrates which might be fermentable, if not by yeast, then perhaps by bacteria. It is very questionable, however, whether such fungi would be able to break down lignified cellulose into these carbohydrates at a sufficiently rapid rate for the method to be adopted in technical processes.

**The production of organic acids from hemicelluloses and cellulose.**—In yet another direction, in the production of organic acids, it may one day be found possible to utilize the microbiological decomposition of hemicelluloses and cellulose for the production of industrial compounds. Reference has repeatedly been made to the presence of acetic and butyric acids among the decomposition products of these carbohydrates. The formation of lactic acid in considerable proportions as a result of the destruction of xylan by *Bacterium lactipentaceticum* and allied species was demonstrated by Fred, Peterson, and Davenport<sup>14</sup>. It is still an open question whether any of these reactions can be so conducted as to form the basis of technical processes for the manufacture of these acids.



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